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The Iterative Design of Aptamer-Functionalized Membranes to Enable Detection, Sequestration, and Recovery of Small-Molecule Compounds

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The Iterative Design of Aptamer-Functionalized Membranes to Enable Detection, Sequestration, and Recovery of Small-Molecule Compounds

By

Misael Adair Romero-Reyes
B.S., Universidad Autónoma del Estado de México, 2016

Advisor: Jennifer M. Heemstra, Ph.D.

An abstract of
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Abstract

The Iterative Design of Aptamer-Functionalized Membranes to Enable Detection, Sequestration, and Recovery of Small-Molecule Compounds

By Misael A. Romero-Reyes

Decontaminating our increasingly scarce sources of fresh water has become of paramount importance. Of the available treatment purifications methods, membrane filtration is advantageous due to its simplistic design, affordability, and applicability. However, one of the biggest drawbacks is its inability to retain small molecules of interest (< 1 μm). This matter becomes more pressing because contaminants arise from multiple industries, human pollution, and natural bacteria. This thesis is focused on exploring the preparation of a filtration system capable of removing small molecules from water by leveraging the use of aptamers. Aptamers are single-stranded DNA that have strong affinity and specificity to small molecules. When we couple aptamers to current filtration systems, we can pave the way to remove small-molecule and macromolecular contaminants, toxins, and microorganisms from water in a user-friendly manner. In Chapter 1, I provide a literature review to introduce the concepts of water scarcity, current technologies and methods for water decontamination, the use of membranes in decontamination, and utilization of aptamers in environmental applications. In Chapter 2, I create the first aptamer-functionalized membrane and demonstrate its ability to remove bisphenol A. In Chapter 3, I explore this system further by optimizing each component of the preparation process and therefore the increase of aptamer attachment. We also demonstrate the depletion of more than one small molecule synergistically. In Chapter 4, I talk about applications of the membrane system developed that go further than removal of small-molecule contaminants from water sources. I evaluate the creation of a membrane that can self-regenerate by not only removing but also degrading a small-molecule contaminant with the aid of an enzyme. In Chapter 5 I reflect about my time in graduate school, where apart from research, I got intensive training on teaching, science communication, and outreach. Finally in Chapter 6 I discuss the practical applications and consider future directions of the system developed. Together, this research sheds a light in the development efficient methods that can sequester and remove micro- and macro- molecules from our water supplies, which due to the imminence of fresh-water scarcity, it has become critical to study.

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Dedications

I would like to dedicate this dissertation work to my family, partner, and many friends.

To my loving parents, Moises and Carmen for all the support provided over the years. Your sacrifice and continuous lessons have helped me pursue my dreams and prepare me to be the leader I aspire to be. Thank you for your unconditional love and support even when we are in different countries, many many miles away. I am the luckiest person alive to have you as parents.

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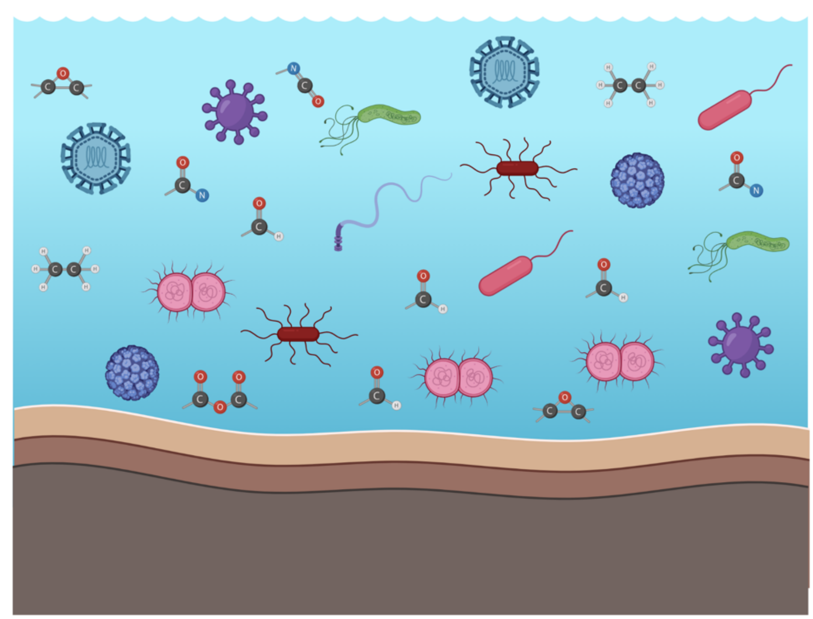
Chapter 1

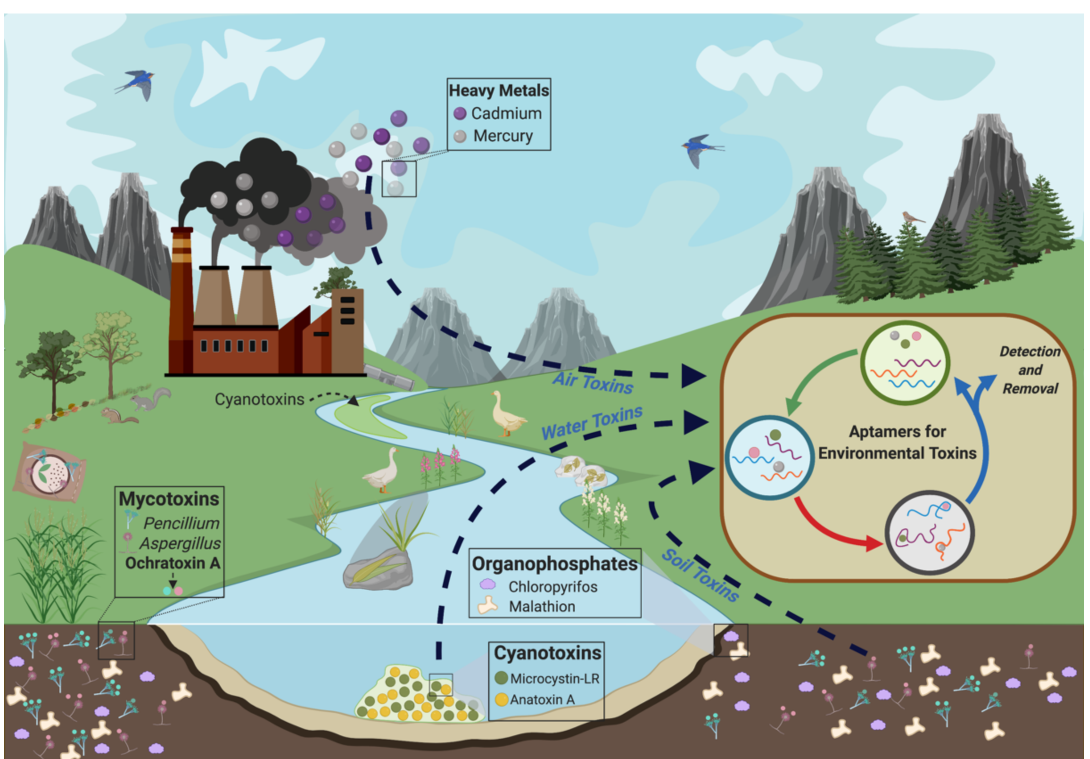
Combatting Small-Molecule Environmental Toxins: Detection and Sequestration using Functional Nucleic Acids.



In this chapter, we introduce the different technologies for water decontamination and introduce aptamers as a viable way to decontaminate water sources when attached to current purification methods.

1.1 Abstract

Small-molecule contaminants pose a significant threat to the environment and thus human health. While effective methods are available for water treatment in developed countries, detection and remediation of toxins in more resource-limited environments and recreational water sources remains a challenge. Functional nucleic acids, including aptamers and DNA enzymes, have emerged as powerful options to address this challenge. The goal of this thesis is to outline recent efforts toward the selection of aptamers and enzymes for waterborne toxins and describe their application for toxin detection and remediation. We will explore different water decontamination technologies and methods used with their limitations, that prompt the use of membranes, a cheap and inexpensive technology that can be enhanced to include the removal of micro- and macro- molecules with the help of aptamer systems. Finally, we will include an outlook that addresses barriers that still exist to widespread adoption of these technologies and propose a path forward toward addressing these barriers.



1.2 Introduction

Unbeknownst to the majority of people, of the available water on the surface of our Earth, only 0.03% of water can be used for human consumption.[1] Due to the rapid changes in human urbanization, pollution, and overpopulation there has been a decrease in water access.[2] This is especially critical since we are approaching a time when there are different cities that have reported complete drought, and many more that are in danger of experiencing scarcity in the coming years.[2-4]

Fresh water is often found in rivers lakes and reservoirs in continental land. This constitutes the majority of the amount of water that can be used for human consumption and unfortunately is often found as a mixed matrix, unable to be consumed right away.[5-6] There are various factors that have caused this over the past few decades including increase in temperatures [7-8] and contamination from various industries,[9] which increases the complexity of the water by the increase in the amount of synthetic and natural contaminants in the water. Synthetic contaminants include small-molecule organics, heavy metals, and polymeric chemicals.[10-11] Biological contaminants include the increase of bacteria and viruses in the water.

Due to the complexity of these water sources, there has been an increase in the research regarding water decontamination. Most of decontamination techniques include the use of physical or chemical treatment, or a combination of the two. Specifically, centrifugation, coagulation, chlorination, and photochemical inactivation are the main techniques used to decontaminate freshwater sources.[12-13] All of these require the use of high amounts of energy, machinery, and complex processes, which makes the overall process expensive.

For this reason, there is a necessity to research more user-friendly and point-of-use systems, which aid in the decontamination of not only macromolecules, but also small molecule toxins and contaminants.[12, 14] To find the correct platform for this application, many considerations must be met: effectiveness, practical application (easily performed by the user), inexpensive, scalable, and sustainable design.[15] Of the current methods available, membranes have been

proven to be efficient. Membranes remove contaminants depending on their physical properties, such as size.[16] Although multiple membranes have been engineered from multiple materials, they face the same limitations as other decontamination techniques: as the pore size gets smaller, the machinery and resources needed to decontaminate water increases. Consequently, we have to strike a balance between price and contaminant removal.[17-18] Given that wider-pore membranes are able to remove microorganisms and macromolecules, and at the same time are still accessible and user-friendly, they are a great platform that can serve as a scaffold for decontamination of water. Their only drawback is the inability to remove small molecule contaminants and toxins (< 1um).[19]

Some functional nucleic acids are single stranded oligonucleotides that have the ability of either sequestering or performing a reaction in molecules.[20] Aptamers are single stranded DNA or RNA molecules that have strong affinity and specificity for small molecules of interest.[21] DNAzymes on the other hand perform a reaction or are able to degrade small molecules of interest.[22] Their ability to recognize and bind small molecules of interest has given functional nucleic acids broad use in multiple applications. These include biomedical applications such as delivery, therapeutics, clinical diagnostics, imaging, and biomarker discovery.[23] More interestingly, there has been an increase of its use in environmental applications such as the detection and the bioremediation of molecules of interest. In view of its innate ability to sequester small-molecule contaminants and toxins, aptamers can be a suitable system to decontaminate water.[24]

In this thesis introduction we discuss the severity of freshwater contamination and the different technologies up to date that decontaminate freshwater together with its limitations. We dive deeper into the different types of membranes prepared up to date and how ultrafiltration membranes serve as an excellent scaffold for our study. We do a deep study on the aptamers used for environmental applications for detection and removal to date and define the types of

molecules we are interested in removing for water. We summarize the objectives that we are trying to accomplish by attaching functional nucleic acids to membranes.

1.3 Earth's Freshwater Crisis

Most of the water in the atmosphere is in the form of saltwater (in the sea) while freshwater accounts for just 1%. Of this 1%, mostly comprised of ice, snow or groundwater, only 0.03% is in liquid form on the surface.[25] Fresh water is considered to be any water body that is not salty and thus is considered suitable for consumption. Freshwater does not only relate to the water itself but also the ecosystems and diversity within it.[26] Because it is a resource for humans, current extraction practices have increased and are huge pressure on these ecosystems. This has had catastrophic consequences in the biodiversity of the world.[27] Human consumption of water is attributed mostly to population growth, economic development, and dietary changes. Almost every activity or device humans use include the use of water in some related or unrelated way, so it is not surprising that these sources are being depleted.[28]

Because surface water is being depleted at such increased rates, people and industries have relied on the use of groundwater as a source for consumption. Groundwater refers to the water available under the soil or rock formations.[29] Because it is not accessible, most of the extraction of this water requires different technologies and methods. These methods include the use of drills to make wells and pumps that bring water to the surface.[30] Because of its native location, most groundwater is susceptible to contamination, mostly due to human activity (extraction, agriculture, commercial, and residential). But, special importance must be paid to contamination due to extraction of ground water because different phenomena can occur such as contamination due to the *zone of contribution*, or *interaquifer leakage*. Regardless of these consequences, most of the freshwater is used in the crops that we grow for human consumption or animal feeding; these activities use the most amount of water with an exorbitant amount of 90% of all freshwaters used

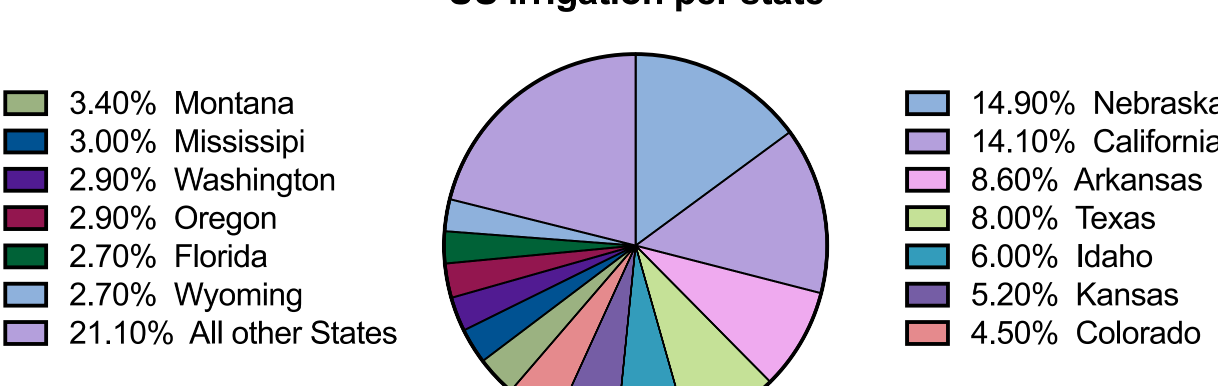


Figure 1.1 State shares of US irrigated acres in the year 2012. Adapted from *USDA Irrigation and water use.*

for this purpose in the US.[31-32] As seen in **Figure 1.1** most of the irrigation happens in the western states, as they are more viable to grow crops due to environmental factors. As population keeps increasing, the demand for water and food will increase as well. It is expected that by 2050 there is going to approximately 10 billion people in the world and the demand for water will increase by 25%, an increase of the already high demand.[33] Multiple agencies and governments have set policies in place to have a greener and more sustainable use of water, but unfortunately, we are at a place where we are depleting sources at a higher rate in which they can be regenerated. The problem relies on the *virtual water*, which is the water used to develop the different products, services, and processes, and oftentimes consumers do not see.[34] There have been various proposed solutions that humans can adopt to alleviate this pressing water usage. One example, being the most important, yet controversial, is the change to a vegetarian diet. It has been shown that if people switch to a vegetarian diet can sustainably reduce water consumption by 27%-41%. This would incredibly increase the water availability in the coming decades.[35-36]

Another thing to consider about water availability throughout our earth is the unequal distribution of it. Some continents, or even states, have more water bodies and annual precipitation than others, leading to a lack of access of water to a numerous amount of people. It

is known that 30% of its current people (population 7.6 billion people) suffer from lack of access to safe drinking water. As a consequence, people drinking contaminated water suffer curable diseases (cholera, diarrhea, typhoid fever, etc.) and are more likely to die. Because of this, the United Nations has set forth a plan to tackle this crisis by 2030: *United Nations' Sustainable Development Goals.*[37]

Because of the current freshwater crisis, scientists have moved towards investigating the potential to reuse water. By decontaminating, that is, removing toxic contaminants from water, it can be reused especially in crops and other non-human intake applications. Special attention has been given to decontaminating the water for human consumption, as this is the most pressing issue in water scarcity.[38] As we move forward, special focus will be given to the different techniques and technologies used in water decontamination in both developed and undeveloped countries. We will observe the current gaps in the decontamination technologies and why the usage of a user-friendly decontamination system is important and of maximum importance in today's day and age.

1.4 Technologies and Methods used in Water Decontamination

Water decontamination has been changing over the last few years. This is because the number of contaminants in water has also been increasing. With the increase of temperature in our planet, there are lower levels of dissolved oxygen, due to their inverse relationship. Because of this, there is an increase in pathogens, nutrients, and invasive species in the water.[39-40] An example of this is the increase in algal blooms, which we have observed a rise of in the last few years. On a chemical perspective, an increase of temperature also increases the concentration of some pollutants, such as ammonia, due to their chemical response to warmer temperatures.[41] The final consequence of an overall temperature increase is the increased rates of evapotranspiration from waterbodies, which result in the shrinking of waterbodies themselves.

Because of the increase in temperature, and pollution involved during human activities, there are four types of contaminants associated with water contamination: inorganic contaminants, organic contaminants, biological contaminants, and radiological contaminants.[42] In terms of *inorganic contaminants*, we often talk about the chemical parameters of the water (e.g. hardness of carbonates). Most importantly we talk about heavy metals (arsenic, lead, copper chromium, mercury, antimony, etc.) that can go into our water sources.[43] In terms of *organic contaminants*, the major sources are pesticides, domestic waste, and industrial waste. These types of contaminants are often the most harmful for humans because they can cause a variety of diseases including cancers, hormonal disruptions, and nervous system disorder.[44] In terms of *biological contaminants,* which are caused by the presence of living organisms include algae, protozoan and viruses. Of these, algae are oftentimes the most harmful because they are able to have excess growth, but most importantly (and critically) their release of toxins are capable of damaging the liver, nervous system, and skin.[45] Lastly, *radiological contaminants,* are contaminants that are derived from radioactive elements, thus are more centralized near water bodies that are close to nuclear industries or near natural deposits of radioactive minerals. The outstanding consequence of drinking radiological contaminated water is the increase risk of cancer.[46]

There is a diversity of the contaminants in our water sources. For this reason, there have been numerous attempts to solve this issue. The suitable technology that can remove all contaminants from water and is affordable has still not been found, although there is a promise if a combination of these technologies can be done in parallel. The common water purification methods can be divided into sedimentation, boiling/distillation, chemical treatment, disinfection, and filtration.[47] We will disseminate the aforementioned techniques by identifying their current status in water decontamination.

1.4.1 Sedimentation

Sedimentation is the most convenient and often first step in water decontamination. It relies on the physical process of using gravity to remove suspended solids from water (**Figure 1.2**). There are often two ways of achieving this 1) turbulence of moving water or 2) naturally in the still water of lakes and oceans.[48] Since this is a time driven process, oftentimes a turbulence

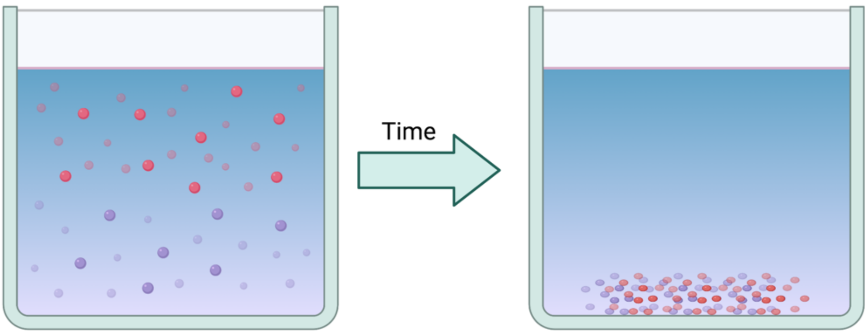


Figure 1.2: Sedimentation process. This process used the physical properties of the different contaminants and gravity to remove suspended solids from water.

in the top to achieve sedimentation faster is opted for. This mechanical assistance is called thickening. This process is often done as a starting point in the decontamination procedure because it minimizes the need for other decontamination strategies such as coagulation and flocculation, in which chemicals are needed. The complexity of this procedure depends on the concentration of the particles to be removed. Oftentimes, small concentrations can get by without mechanical assistance, but as concentration and diversity of particles increase, there are more barriers to achieve sedimentation.[49] Because of this, various specialized tanks have been created, including horizontal flow, multi-layer, radial flow, and settling tanks. Sedimentation is accomplished by a decrease in velocity of the water to a point which they no longer remain in suspension. Out of the techniques and technologies discussed here, this is the least invasive technology, but at the same time removes only visible suspended contaminants that are big enough. This technology is also defined as clarification.[50]

1.4.2 Boiling/Distillation

Distillation is the most common separation technique. This technique relies on the differences of the mixed components when heat is applied. It is based on the differences in their boiling points. Because of this it is dependent on the vapor pressure characteristics of the components present. It is most commonly used in the separation of water and inorganic substances such as lead, calcium and magnesium. Because of the increased temperature it can also destroy bacteria. Due to the innate characteristic of water having a boiling point of 100 °C, organics with lower boiling points than that cannot be removed effectively from water and become even more concentrated. Because of this, oftentimes distillation has to be coupled with a carbon filter or some other technology that can tackle organic contaminants. Apart from the inability to

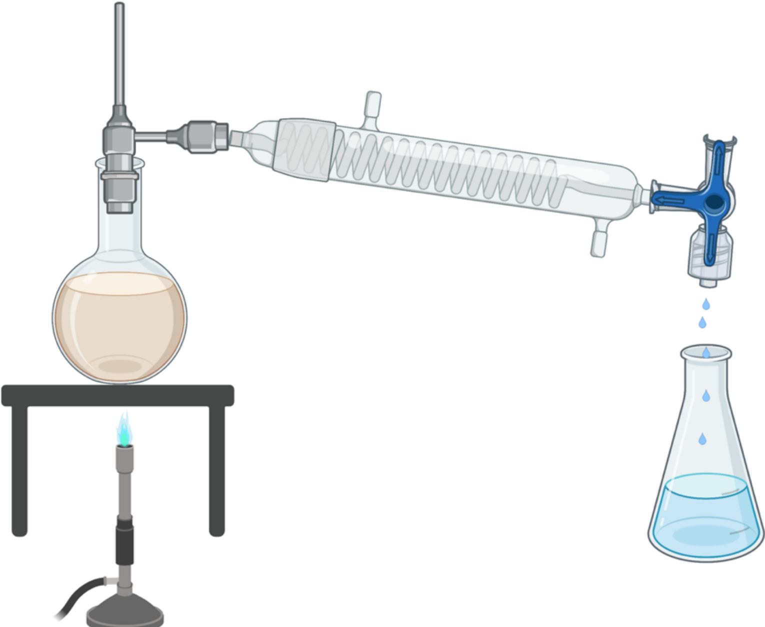


Figure 1.3: Distillation procedure. Mixed components are separated by the application of heat and recondensed in a separate container.

remove some organic contaminants, another concern of using distilled water is that although its safe, it does not have nutrient minerals for drinking water purposes. In summary, the benefit of this technology is that it can remove a broad range of contaminants, is continuous, does not require the use of additional processes or the use physical barriers. However, this technology

consumes an enormous amount of energy (heating and cooling), some contaminants remain in the condensate, requires careful maintenance, and is not as effective with lower volatility organics.[51-52]

1.4.3 <u>Chemical Treatment</u>

Oftentimes different chemicals are added to the water to promote the removal of contaminants (precipitation and coagulation) or are attached to a surface or a column (adsorbents) to promote trapping of different contaminants.[53] The use of different chemicals is important as they accelerate the decontamination process. A lot of care must be taken when deciding which kind of chemicals should be added to the water, as these can also give rise to by-products, which further contaminate the water. Most of the times, the best method of control is through management practice and optimization of chemicals that come in contact with water, rather than through monitoring chemical analysis. In this section, the most important processes dealing with chemical treatments will be discussed.

1.4.3.1 <u>Precipitation</u>

Precipitation is a process in which one or more substance is removed from a solution by adding reagents so that insoluble solids disappear. It is based on the solubility rules and ion concentrations in solution. Because precipitation occurs between molecules in the water, it is considered one of the simple methods to purify water.[5] These chemicals usually form particles which settle at the bottom and can often be dewatered and disposed of. This technique is also used for softening the water (removal of inorganics: Ca/Mg in terms of carbonate, bicarbonate, chloride and sulfate), removal of heavy metals, arsenic, phosphorous fluorides and dyes.[54] Overall, precipitation is a simple process, which is effective for the removal of multiple heavy metals and can be applicable to the removal of natural organic matter and dissolved organic carbon. Albeit these great benefits, there are some concerns in using precipitation as a water

Softening of Water	Removal of heavy metals
Addition of $Ca(OH)_2$	$H_2Cr_2O_7 + 6FeSO_4 + 6H_2SO_4 \rightarrow Cr_2(SO_4)_3\downarrow + Cr_2(SO_4)_3 + 7H_2O$
$Ca(HCO_3)_2 + Ca(OH)_2 \rightarrow 2CaCO_3\downarrow + 2H_2O$	$Cr_2(SO_4)_3 + 3Ca(OH)_2 \rightarrow 2Cr(OH)_3\downarrow + 3CaSO_4$
$MgSO_4 + Ca(OH)_2 \rightarrow Mg(OH)_2\downarrow + CaSO_4$	**Removal of fluorides**
Addition of Na-aluminate	$2HF + Ca(OH)_2 \rightarrow CaF_2 + 2H_2O$
$MgSO_4 / Cl_2 + NaAl_2O_4 + 4H_2O \rightarrow Mg(OH)_2\downarrow + Na_2SO_4/NaCl + 2Al(OH)_3$	$CaCl_2 + 2HF \rightarrow CaF_2\downarrow + 2HCl$
Heat	
$Ca(HCO_3)_2 + heat \rightarrow CaCO_3\downarrow + H_2O + CO_2$	

Figure 1.4: Common precipitation techniques. Most of the components removed are inorganic based contaminants. This table is adapted from reference 5.

decontamination technique. First and foremost, it requires a continuous supply of chemicals which in turn produces a huge amount of by-products that need to be handled and disposed of correctly.[55] The most common removal techniques using precipitation can be found in **Figure 1.4**. Oftentimes, the disposal of the precipitation sludge is a concern because it ends up in landfills that directly or indirectly connect to freshwater waterways and contaminate all over again.

1.4.3.2 Coagulation and Flocculation

Coagulation is a process in which the addition of a "coagulant" is added to destabilize a charged particle. Oftentimes this technique is coupled with flocculation, which is a physical technique that promotes agglomeration and setting of the particles by mixing. Although pretty similar to precipitation, the main difference between this technique and precipitation is that precipitation is carried out on a single-phase system, while coagulation and flocculation in two or more phases. Coagulant chemicals often have charges opposite to those of the suspended solids, which neutralize such non-settable solids and allow them to be deposited to the bottom of the chamber. After coagulant chemicals are added, a gentle mixing stage is carried out that increases the particle size from submicroscopic to visible suspended particles. These suspended

agglomerates then settle on the bottom of the tank and are able to be removed, so that this process can be carried out multiple times.[56] Oftentimes this process is used as a first step in the treatment process because most of the particles are removed and the water is clarified. This way, subsequent steps (e.g. chlorination, a disinfection technique discussed later), will require less

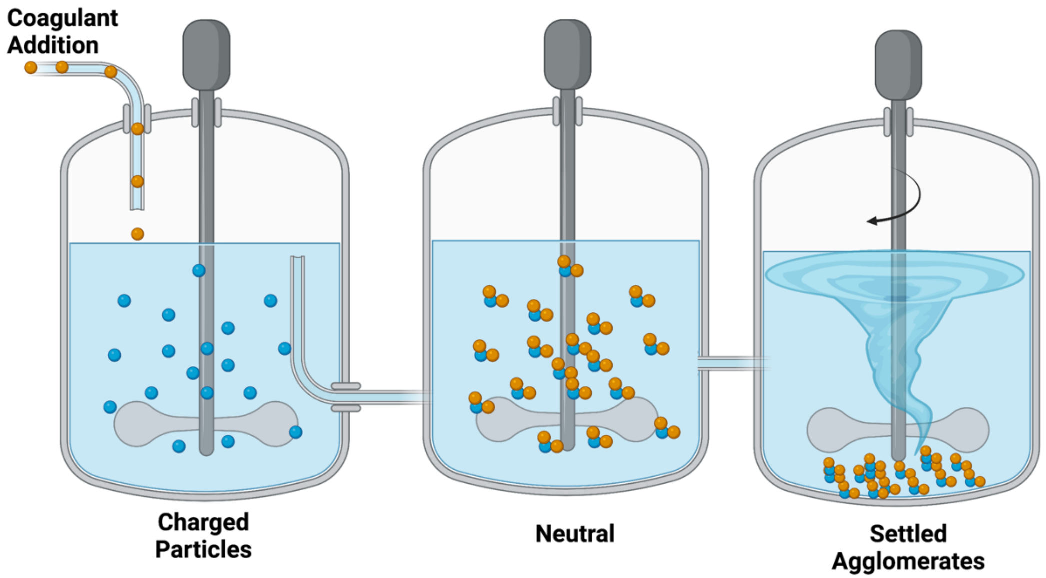


Figure 1.5 Coagulation-Flocculation process. This process is based on adding coagulant chemicals to contaminated water that bind to charged particles forming neutral agglomerates.

usage of resources. The complete process of coagulation-flocculation is described on **Figure 1.5**. There are two types of commonly used coagulants and they often fall into two categories: ones that are Al based, and ones that are Fe based.[57] Aluminum sulfate, aluminum chloride and sodium aluminate are common coagulants of the former group while ferric sulfate, ferrous sulfate, ferric chloride and ferric chloride sulfate are the most common in the latter category. Although these inorganic coagulants constitute the most common coagulant used, oftentimes organic coagulants are also used. Organic coagulants, which are generally used for solid and liquid separation and sludge generation include the use of polyamines, polyDADMACs, melamine formaldehydes and tannins. The first two act as cationic neutralizers while the latter two absorb organic materials such as oil and grease. While the inorganic coagulants are most cost effective, scientists have

opted to do combinations of both (organic and inorganic) to achieve the advantages of both coagulants and decrease the amount of chemical added to the water that is meant to be treated.[58]

1.4.3.3 Adsorbents

This is another physical process in which dissolved contaminants adhere to a surface, usually porous, containing solid particles. It is a physical outcome of surface energy, that bases itself on the Van der Waals attractive forces that pull the solute of the solution and into the surface. Oftentimes, the absorbent systems are added directly to the water supply or mixing throughout. Unlike the previous methods discussed, this method has good processing effect, low cost, and less secondary pollution to the environment. This process relies on various materials to absorb different contaminants. These include activated carbon, activated, alumina, chitosan, zeolite, and clay minerals.[59] While these materials all perform similar activities, the difference relies in the types of contaminants they remove from water. The major differences are as follows:

Activated Carbon. Activated carbon is the most common type of adsorbent and is similar to the common charcoal. It is so typical that it is often found in households in different products and processes. It is also considered the oldest type of adsorbent up to date. It is composed of a porous surface void and allows the adsorption of heavy metal ions, with a surface area that can reach the 500-2000 m^2/g. To achieve the high porous formation, the use of high heat (1300 °C) in carbon is performed in the presence of inorganic salts. This carbon source can be derived from petroleum coke, bituminous coal, lignite, wood products, or coconut/peanut shells. Usually, the generation of steam and evacuation of gas from the carbon structure increases their surface area and therefore, their decontamination efficiency. Although most of the time heat is the only reactant in this process, the addition of activating agents such as phosphoric acid or zinc chloride can be added to accelerate this process. The only drawback of using activated agents is that then the activated carbon then must be washed with water. Up to date, there are two different forms of activated carbon; granular activated carbon and powdered activated carbon. The main difference

between them is their reusability. While the granular activated carbon can be regenerated, the powdered one is too small to be reused. Overall, activated carbon is an effective method to remove carbon-based impurities, chlorine and odors. It is also very cost effective and has a high capacity due to the increased surface area. Their limitations include the ineffectiveness of contaminant removal because of “channeling” (reduction of contact contaminant and activated carbon), which in turn can lead to accumulation of bacteria in the filter. Also, they don’t have a long lifetime as they need constant filter changes.[60]

Activated Alumina. Activated alumina consists of Al_2O_3 beads that are highly porous and exhibit a high amount of surface area. Although a bit lower than activated carbon (345 - 415 m^2/g), this material does not soften or disintegrate when immersed in water. It is mainly used in one of its three forms: activated alumina sorbent, activated alumina desiccant, and activated alumina catalyst carrier. One of the most important benefits of using activated alumina is that it can be tailored by varying the activation process and dopant variation. It is able to remove many heavy metals (Se, SB, Pb, Bi) and ions (As^{5+}, PO_4^{3-}, Cl^-, F^-). The main drawback of this technology is that it often needs to be coupled with another technology because it cannot remove most contaminants of health concern.[61]

Chitosan. One of the biggest benefits of using chitosan as an adsorbent is its low cost and its very strong adsorption capacity to heavy metals. Chitosan is considered to be inexpensive because the fish waste source is cheap. Chitosan is used as a flocculant, which aids with the turbidity of the contaminated water. Chitosan is derived from Chitin, one of the worlds larges biological polymers. Chitosan has been used to remove Cd in very high yields. Chitosan is also able to remove other heavy metals like Hg^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+}. Currently the trend is going towards the chemical modification on Chitosan for a better removal of heavy metals. Because chitosan is widely available, experimentation can be done in it to make sure that the removal capacities of adsorption of different contaminants increase.[62]

Zeolite. Zeolite is a common mineral and adsorbent, often composed of aluminosilicates with an Si/Al ratio 1:∞. Composed of a tetrahedral network of Si and O atoms (which then get substituted with Al), the adsorptive property is due to the crystalline nature of this material. The structure of zeolite has a lot of space, which can in turn undertake ion exchange with another metal ions. Zeolite materials have proven to absorb a variety of heavy metals (Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}). Zeolites have found to have a surface area of 1-20 m^2/g. High temperatures can improve the adsorption capacity of these kind of materials. This have given rise to the production of synthetic zeolites that are manufactured by hydrothermal processes in a temperature range of 90 °C -100 °C. Zeolites have been found useful for many applications most which rely on the easy exchange of the cations withing its structure that makes it feasible to be reused. It has been shown to also remove radionuclides, organic, humic substances, and microorganisms. One of the biggest drawbacks of using this material is that they lead to an increase in sewage sludge mass because the material softens after time.[63]

Clay Minerals. Because coal comes from peat, lignite, etc., they are rich in humic acid, which in turn can be processed as an adsorbent. Clay minerals are mainly composed of hydrous aluminum silicates that contain significant amounts of iron, alkali metals, or alkaline earth metals. These properties give them the ability of having neutralizing applications in water treatment. Research is moving toward the synthesis of multi-functional modified clays that have large surface area, high porosity, high acidic sites, non-toxic, easy filtration, and regeneration.[64]

All of the aforementioned absorbents have different characteristics and therefore their depletion capacities are different depending on their internal and external structure. Most of the research within the area of absorbents is focused on the modifications that can be performed in the different materials that would enhance their contaminant depletion. Most of these have to be used in parallel with other technologies or describes, as they are not able to remove all contaminants from water, and are mainly focused in the removal of heavy metals.

1.4.4 Disinfection

Disinfection methods are often divided into two categories: chemical and physical methods. Physical methods include UV, solar radiation, and ultrasound, while chemical methods include chlorine, iodine, and ozone.

Ultraviolet Radiation. In this type of water treatment, the water passed through a UV light (acts as a germicidal), which damages the genetic components of the microbes. The major drawback of this water treatment is that it is ineffective in removal of dissolved organics and other particulate matter, especially in water of high levels of suspended solids. Benefits of this technology include the ability to destroy many microorganisms, minimal effects on the minerals in the water, and the ability to degrade some organic contaminants, all of this while no additional toxic and non-toxic chemicals are introduced. An additional drawback of this technology is that it relies on the use of electricity, without it, it cannot be possible.[65]

Solar Radiation. Acts in a similar fashion as UV, it inactivates pathogens, but it requires to be under the sun for at least 6 hours for it to let the UV-A radiation of the sunlight destroy such pathogens. This technology is the easiest to use and is inexpensive since there is no necessity other than sunlight. Although it has good bacterial and viral disinfection, it is dependent on climatic conditions, and does not ensure the removal of bacterial spores and cysts of some parasites.

Ultrasound. Ultrasound is the mechanical vibration of the waves that can be used to damage the cellular structures of the bacteria. Although this is a useful way to disinfect water, the regrowth of microorganisms is possible. For this reason, the combination of this and a chemical disinfectant is optimal.

Ozone. Ozone is an unstable form of oxygen that makes a powerful disinfectant due to the formation of radicals. It readily gives up oxygen making it a powerful oxidizing agent. Ozone oxidizes the organics in the bacterial membrane which caused cellular rupture. Ozone is also used to improve the clarity of the water because Fe (II) and Mn (II) are transformed into the insoluble Fe (III) and Mn (VII), which allows for a subsequent filtration. One of the main

advantages of ozone is that no residual disinfectant is left in the water solution. This is because the ozone is generated and applied on site. The main concerns of using ozone as a disinfectant is that it is a significant air pollutant, explosive and has various symptoms to the person that is drinking this water. It is also believed that it can produce carcinogens.[66]

Chlorination. The most common and strong oxidant is chlorine and derivatives. It treats against bacteria and protozoa in the water that form cysts. Since the gas is dangerous to use, most common chlorine forms appear in the practice of sodium and calcium hypochlorite. One of the biggest limitations is that chlorine reacts with natural organic compounds in the water and forms potentially harmful chemical byproducts, which cause cancer. Because of this it is preferred to use after another decontamination method like coagulation or sedimentation. Furthermore, the excess of chlorine produces a characteristic taste that has an irritating effect of the mucus membrane of the drinker.[67]

Iodine. Iodine is also a good oxidizing agent. It is good to remove pathogenic organisms, spores, cysts and viruses. Its mechanism relies on the formation of *N*-iodo compounds as a way of inhibition of protein function (reacting with the -NH functions of amino-acids). Their benefits include the effectiveness against many pathogenic organisms, while eliminating the chances of deficiency of iodine. The major drawback of using iodine is that higher concentrations are needed (than chlorine for example) and is more costly. In an aesthetic sense, the use of iodine oftentimes changes the color of the water to a darker color, which can be perceived bad to the consumer.

The disinfection procedures outlined above, both physical and chemical, focus on the removal of microorganisms. While they are very good at that, the complexity of the water sources oftentimes has other contaminants within it, like inorganic, organic and heavy metals, which makes it quite difficult to use these technologies as a stand-alone process. The use of other technologies is important to achieve a decontamination that is optimal for the consumer of such drinking water.[68]

1.4.5 Filtration

Filtration is a technology in which water flows through a filter designed to remove particles from within it. Filters can be made from a variety of sources including sand, gravel and crushed anthracite. These filters have to be routinely cleaned by backwashing. Removal takes place by different mechanisms including straining, flocculation, sedimentation and surface capture. And they are categorized by the method of capture which include exclusion of particles at the surface, or deposition within the media. Strainers are mostly made of metal or plastic and are composed of a simple thin physical barrier, which removes larger contaminants from water (1- 10 cm). Filters, consist of a medium in which most of the particles will be captured. They often come ad disposable cartridge filters, precoat filters (with coating of diatomaceous earth).[69] Granular media filters are used in two different ways: slow-sand filtration and pressure filtration. Filtration depends on a combination of physical and chemical processes, the most important being adsorption.

One of the most important an innovative ideas of water treatment is the use of membranes. Oftentimes a semi-permeable membrane is used to remove impurities. There are various kinds of membranes but are often divided into two categories: pressure driven, and electrically driven.[70] A broader section on the different types of membranes used for water purification can be found in section 1.5.

1.4.6 Limitations of current water decontamination technologies

As it can be seen through all the different technologies discussed thus far, there is not one that can remove all the contaminants at once. Some use increased amount of electricity while others that are more cost effective are not ideal due to low yields of depletion. Current water treatment facilities have opted to use a combination of these technologies and use them in tandem to achieve the desired decontamination of water.

Another factor to consider is the type of contaminants that the different technologies remove. While adsorbents are focused on inorganic and organic contaminants, disinfectants are focused

on the degradation of microorganisms. Coagulation and flocculation are also mainly focused on the removal of inorganic contaminants and heavy metals. Due to the diversity of the different water matrices, ample research needs to be done to find a technology that is viable to decontaminate water.

Currently as some places have the technology and resources in place to remove most contaminants from water by using a combination of these technologies, scientists have shifted towards the removal of most contaminants in one go, in a user-friendly manner that is inexpensive. Fortunately, some types of membranes are able to achieve this goal, and although more research has to be done in the area, membranes show a lot of promise.

1.5 Use of Membranes in Water Purification

More than 50 years ago, membrane technology was emerging as a treatment for water decontamination, although their usage was not seen predominant until the emergence of high-performance synthetic membranes.[71] From this time on, most of the research has been focused on the usage of new materials or various configurations of them. Recent innovations include the different analytical and fabrication tools that are surfacing nowadays. The main benefits of using membranes for water purification is their ability to control size regime, performance, transport, and separation. The importance of using membranes for water treatment is the use of their distinct advantages, which include high water quality with easy maintenance, stationary parts with compact modular construction, low chemical sludge effluent, and excellent separation efficiency.[72] A lot of importance should be given to the compact modular construction ability, because this allow scientists to have a variety of membranes with different size, to address any specific needs a community might be facing. For this, reason membranes have been used for a variety of applications including human use, ecosystem management, agriculture and industry, which makes them adaptable to any purpose.[73]

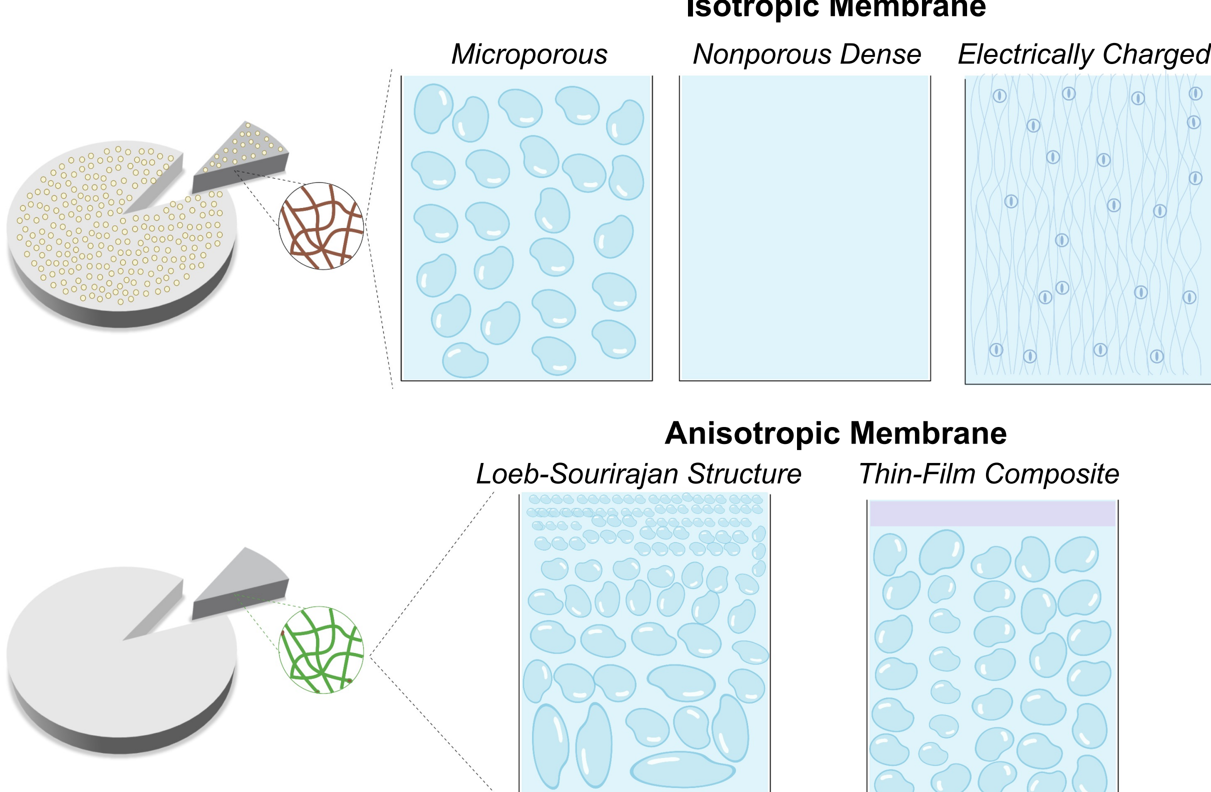


Figure 1.6: Classes of membranes. Isotropic membranes have a homogenous composition, while anisotropic membranes have an heterogenous composition.

Membranes are defined as a thin physical interface that allows certain species to pass through depending on their physical or chemical properties. All membranes can be divided into one of two categories: isotropic and anisotropic. These denominations can be observed in **Figure 1.6**, which depend on the cross-section of the membrane.[71]

While isotropic membranes have a homogenous composition, they can be porous, non-porous or electrically charged. On the other hand, anisotropic membranes are heterogeneous in composition and can have different chemical or physical compositions. Porous membranes separate solutes based on the on the size of the particulate and size of the pore. Just like conventional filters (1-10 μm in size), microporous filters tackle contaminants on a smaller size regime (range 0.1 to 5 μm). The composition of these types of membranes varies, but they are typically polymer-based: track etched, phase inversion, or stretched polymer films. In the case of

nonporous membranes, decontamination takes place because of the diffusion of the contaminants by pressure, concentration or electric-field gradients. Electrically charged membranes can be either microporous or non-porous, but they specialize in the removing charged particles (positive or negative).[74] In terms of anisotropic membranes, Loeb-Sourirajan structures are chemically made of the same material, but their pore sizes differ across the membrane thickness. Finally, composite membranes are chemically and structurally heterogeneous and differ by the different polymers they are made up of. They also include different method of preparation which include interfacial polymerization, solution coating, and plasma polymerization.[75] Typical polymeric materials can be found on **Figure 1.7**. Although most of the

Typical Membrane Materials	
Cellulose acetates	Polyamide
Polyacrylonitrile	Polycarbonates
Polyetheramides	Cross-linked polyether
Polyethersulfones	Polyvinylidenefluoride
Polypropylene	Polyisoprene

Figure 1.7: Typical membrane materials used in membrane preparation. They span from natural-sources to synthetic-made polymers.

materials can be divided into natural polymers, synthetic polymers and inorganic polymers. The most common include the use of synthetic polymers as they have been found a range of application because of its versatility of modification. They also have and increased glass transition temperature, meaning that they can resist high temperatures without compromising performance.[76]

Apart from its chemical and physical composition, the most used categorization of membranes relies on the membrane pore size and therefore the type of contaminants they can remove. They are mainly divided into reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), microfiltration (MF) and particle filtration, in order of increasing pore size respectively.[77] Although all membranes can be anisotropic or isotropic, the removal of the contaminant is what matters and what sets forth using one membrane or the other. As the pore size decreases, the capability of removal increases,

but this does not come without sacrifices. As ymore contaminants are removed, the possibility of getting clogged increases.[78] Figure 1.8 summarizes the common type of contaminants removed

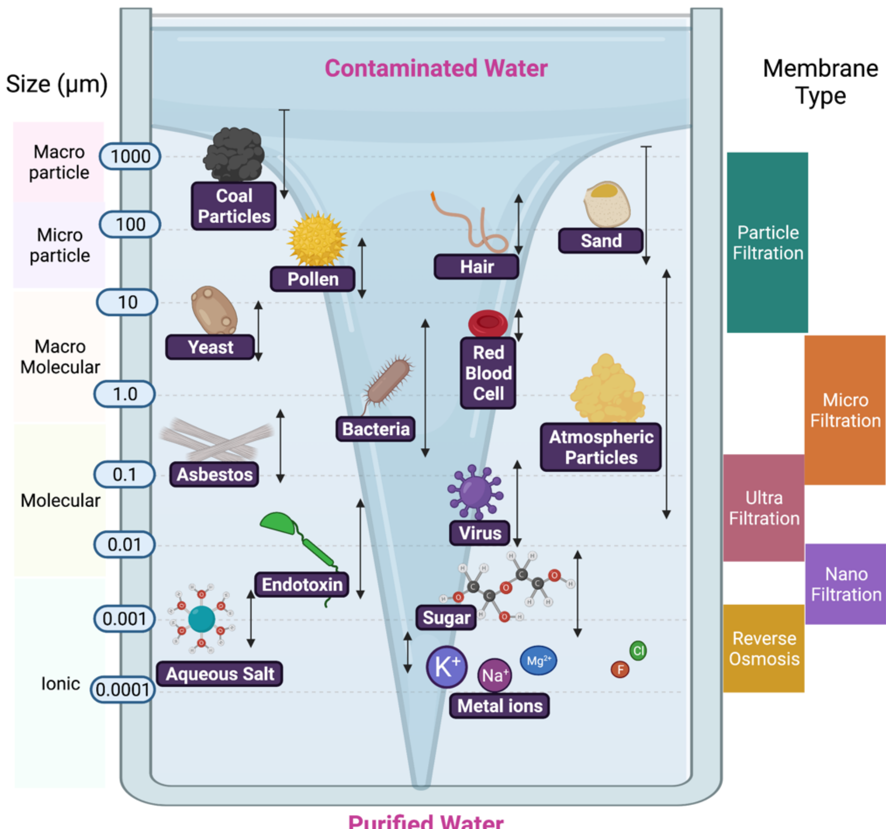


Figure 1.8: Variety of contaminants that can be removed from water depending on the membranes core size. This figure has been adapted from reference 71.

depending on the membrane that is used. As it can be observed, it is not until the use of microfiltration and ultrafiltration membranes that main contaminants can be removed from water. For this reason, the most commercial and available membranes are nanofiltration, ultrafiltration, and microfiltration and are made from synthetic polymers. Although UF and MF are prepared from the same types of materials, the main difference relies on their preparation techniques to afford a variety of pore sizes. Organic polymers are the most common type of material when preparing a membrane, but they can also be made from inorganic materials (e.g., *zeolites: section 1.4.3.3*) that act as good adsorbents.

Inorganic Materials. Inorganic membranes have just recently received attention because of their adsorbent properties and chemical robustness. Some inorganic membranes are able to be reused because of the type of material that is encountered within it. For example, ceramic membranes are prone to exhibit greater fouling resistance than other polymeric membranes. Ceramic membranes include the use of a variety of metal oxides or sometimes composites (containing more than one metal oxide). These types of membranes can even have photocatalytic materials that increase their decontamination functionality. This is because a variety of oxidative and reductive reactions take place on the surface of the membrane. This has the ability of removal of microorganisms (disinfection) and the removal of targeted organic pollutants.[79]

One of the main drawbacks of using this kind of technology is that the requirement of UV light or other excitation source is required so that the membrane functions properly. So, current research in the area of inorganic membranes includes the study of broadening the adsorption spectra of the materials so that this process can be done with sunlight or other less expensive sources.

Although the use of ceramics is the most common type of material used in inorganic membranes, special attention has been given to the use of SiC as a support. This is because nanocrystals can be added to the surface in a stable fashion to remove molecules of interest. Finally, there is also an interest in adding nanoparticles to the membrane to remove biofilms. Ag NPs are the perfect example of this, as they can be added to the surface of the membrane and/or the pores and reduces biofilm adhesion resulting in a strong antimicrobial activity.[80]

Most recently, research has shifted towards the use of graphene-based materials that are able to offer mechanical stability, tunable physicochemical properties and well-defined pores. This add another layer of benefits, as it can be electro- and magneto- controlled for a variety of applications.[81]

Organic Materials. Membranes made from organic materials have all come from polymers. These types of membranes are the main ones used in water filtration technologies due to its ability to offer a variety of structures and properties. The common structures of these polymeric membranes can be found on **Figure 1.9**. They are mostly comprised from synthetic

Figure 1.9: Most common used polymers in membrane formation. These materials are usually derived from natural sources or synthetic sources.

polymeric materials, although one of the most prominent comes from a natural source: cellulose. Depending on the type of membrane needed, the right material must be chosen. For example, for ultrafiltration membranes, PES and PSU are the most common polymers used. They offer great permeability, selectivity of the permeate, mechanical stability, and chemical resistance. Most importantly, these scaffolds offer the ability to be modified, to further enhance of attach other polymers with different systems. For microfiltration membranes polypropylene is the most common polymer used. One of the drawbacks of polymeric membranes is their inherent hydrophobicity, which in turn makes a high fouling tendency which leads to more operational costs

and shorter performance. No single polymer described in **Figure 1.9** has the ability to exhibit the desired chemical/thermal stability, oxidation/pH resistance and mechanical strength. For this reason, research has shifted towards surface modification to make the surface of the membrane more hydrophilic. This includes the use of homogenous blending, plasma treatment, surface grafting, cross-linking, gamma ray and UV irradiation, surfactant methods and surface coatings. Apart from making it more hydrophilic, other factors that are being considered for modification include surface roughness/morphology, pore size, and surface charge. This allows us to make tunable membranes that can address the needs of many scientists and consumers. The use of temperature-controlled filtration has also been studied, which aids on the removal capacity of contaminants from water, and the polymers on the membranes being thermo-responsive allow this to happen.[82]

Although most of the membranes used in current technologies focus on the use of either organic or inorganic materials, attention has been given to the use of inorganic-organic hybrid materials. A combination of both technologies might be the answer to achieve a membrane with the desired properties for water decontamination. As we will see in the future chapters, we try to make a composite membrane, composed of organic-biological materials, to address some of the complications that currently are faced in water decontamination.[83]

1.5.1 <u>Effect of Pore Size and its Limitations to Filtration</u>

The pore size of a membrane is often overlooked at when talking about different filtration techniques. Although generally divided in different categories based on pore size, the membranes are formed of anisotropic identity, so makes it quite difficult to have precise information on their pore size and pore size distribution. Because most of the membranes are prepared by the phase inversion technique, the performance is often a good parameter of the type of the membrane being characterized. The characteristics of membrane pore structures: pores size, pore size distribution, pore density and surface roughness, should be the factors to consider when

fabricating such membranes. There are many techniques for characterizing pore size and are often based in microscopy techniques: scanning electron microscopy, transmission electron microscopy and atomic force microscopy. All of these give characteristic identities of the membrane it is worked with.[84]

The pore size is considered one of the most important factors when filtering water. This is because the pore size will determine the ease with which water can pass through the membrane. As pore size decreases (RO and NF membranes) the machinery, pressure, and cost of filtering water increases. When looking for a suitable water purification system, there needs to be a holistic analysis of what kind of factors are of most importance and how efficient we want the water purification to be.[85]

UF membranes offer some user friendliness in terms of usage, but the main drawback is its inability to remove smaller contaminants of interest. UF membranes do not need special machinery and are able to remove microorganisms effectively. Some tune-in can be afforded in terms of the method of formation and the type of additive that is added. The pore-forming agent in ultrafiltration membranes can be changed and modified to obtain membranes of a certain pore size. This can be advantageous for many reasons, as it will determine the overall characteristics of the membrane and its ability to decontaminate water.[86]

In the future chapters, we will observe how we take an ultrafiltration membrane and modify it to meet specific needs for water decontamination. Most importantly, since ultrafiltration membranes can decontaminate water without the use of resources, they are the perfect scaffold to make our studies in.

1.5.2 Cost and Availability of Water Decontamination Technologies

When thinking about using any technology for water decontamination purposes, one major concern is the cost of such technology. There has to be a holistic analysis of the contaminants that must be removed from water, as well as what resources are available. As seen throughout this chapter, there is a variety of techniques that can be used, and it all depends on the type of contaminant that is set forth to remove. Different techniques specialize in a different type of

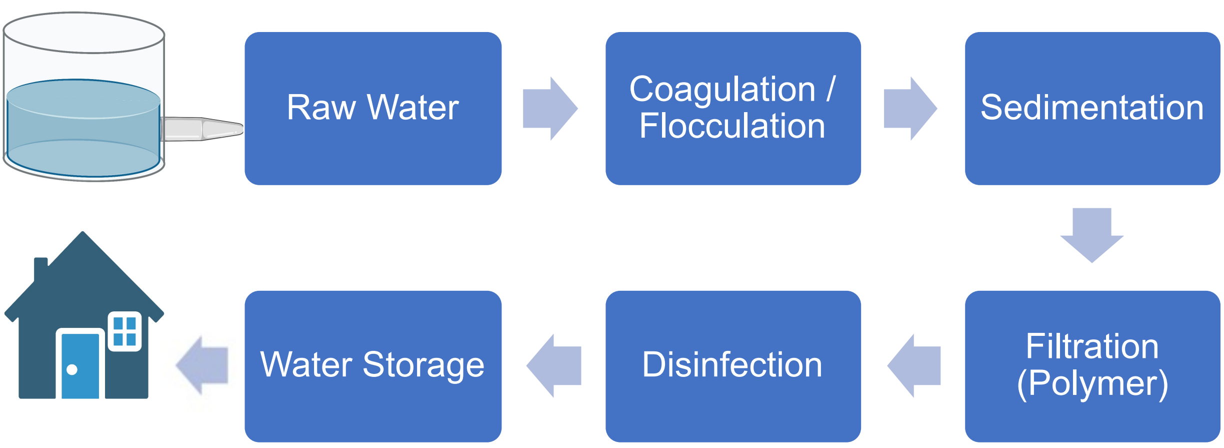


Figure 1.10: Combination of different water treatment technologies that are used in tandem to achieve household drinking water.

contaminant, so it makes sense to use a combination, if not all, of these technologies.[87] That is of course, if there are enough resources for such decontamination event to happen. Most of the developed countries opt to use a combination of different technologies in tandem to purify their water. The most common process can be observed in **Figure 1.10.**

Although the process seems pretty straightforward and has been optimized over the years, there is still a concern that this can only be achieved when there are enough resources to have each of these technologies happen in tandem. There are currently many people in need of access to clean water that unfortunately do not have the resources to have all of these technologies

happen in tandem. For this reason, there should be a special focus on technologies that can remove most of the contaminants in one-go. Membranes have proved to be efficient at this since they are able to remove contaminants depending on their pore size. RO membranes are even able to remove salt from water, which increases the availability of water to be drank since most of the water in our planet is salty. Again, as with every technology, there must be a fine line between the removal of contaminants, and how much it costs. In terms of membrane filtration, the cheaper technologies come from the organic polymers, as they are widely available and modular.[88]

Ultrafiltration membranes show a great promise in terms of affordability, ease of use, and preparation. But the main drawback is its inability to remove small molecule contaminants. Therefore, it must be enhanced to do that, and we are able to show it by using aptamers, exceptional functional nucleic acids that have affinity to a variety of contaminants of interest.

1.6 Aptamers and DNAzymes for Detection and Sequestration

Human exposure to potentially hazardous chemicals is inevitable because of the sheer volume of chemicals reported in commerce, which is expected to drastically increase by 2050. Environmental toxins can be divided into two categories: in soil and in water, although they can be found in indirectly derived from these categories, like for example food.[89]

Many diseases are caused by environmental toxins, thus highlighting that rigorous characterization is needed to mitigate potential health risks. Small molecule environmental toxins can occur naturally, such as cyanotoxins from bacteria and mycotoxins from fungi. A major source of exposure is through drinking contaminated water.[90] Exposure rates largely fluctuate with climate and water purification access in different regions. Similar routes of contact are also prevalent for small molecule environmental toxins that have man-made or synthetic origins. This is a result of large-scale industry practices that meet the increasing demands of modern culture. A prominent example is the use of pesticides, which revolutionized the agricultural field, but has

led to cases of human poisoning from acute and low-level exposure due to their persistence in soil, water, air, and food.[91] The increasing amount and diversity of environmental toxins causes an increase in human exposure risk. In response, agencies around the globe generated guidelines to alleviate the public health risk. These include setting parameters such as tolerable daily intake levels to report on what the maximum amount of an environmental toxin that is considered safe.[92]

Recent efforts at designing adaptable methods for detection of intact toxins in environmental media present a promising new avenue for risk assessment. Nucleic acids have different affinities and catalytic characteristics towards a variety of molecules, which make them the key elements in a variety of different assays for sequestration and detection. Aptamers are single-stranded DNA or RNA molecules (**Figure 1.11**) that can bind to specific small-molecule or protein targets with

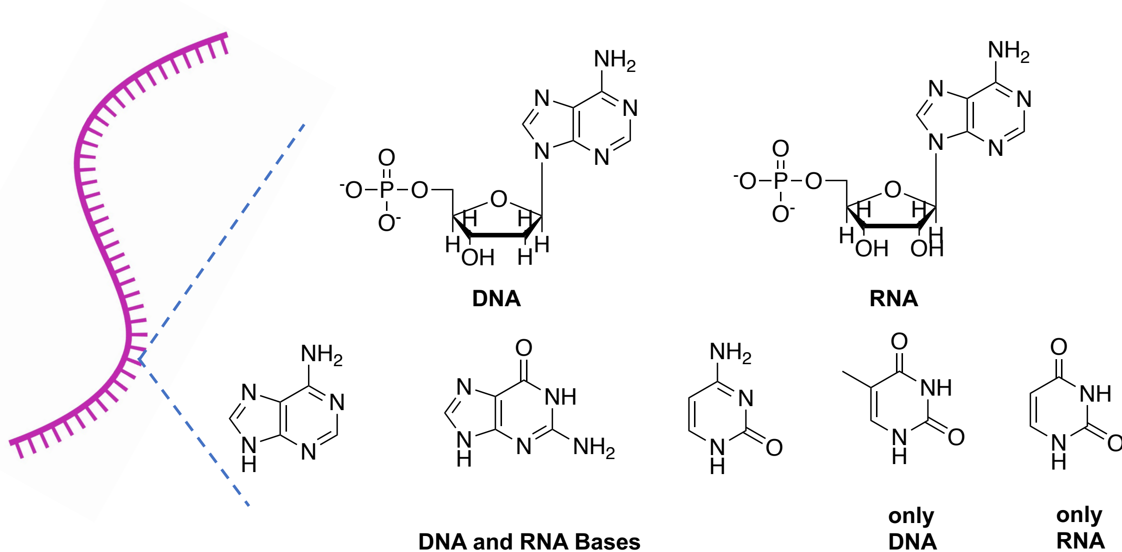


Figure 1.11: DNA and RNA structures. Common bases found in aptamers and DNA zymes.

high affinity and selectivity.[93] Current applications of aptamers focuses on its use in biomedicine and molecular imaging. Applications expand beyond those used in biomedicine. Molecular recognition properties of aptamers have been harnessed for sensing applications. For instance, aptamers can be used as signal molecules in absorbance or fluorescent assays due to some

structural change upon target binding.[94] Encouragingly, there are reported aptamers for various environmental toxins, including both natural and synthetic ones. Considering their sequestration and catalytic properties, they can be used for its use in detection and remediation of toxins. The rest of this chapter will summarize the recent significant efforts in the detection environmental toxins using aptamer-based approaches. Further, we will explore the recent advances in sequestration of these toxins using aptamers.

1.6.1 Selection

Aptamers are generated by Systematic Evolution of Ligands by Exponential Enrichment (SELEX), a process in which a library having a large (10^{12}-10^{14}) number of sequences is incubated with a ligand and sequences that show the desired binding activity are enriched over multiple rounds.

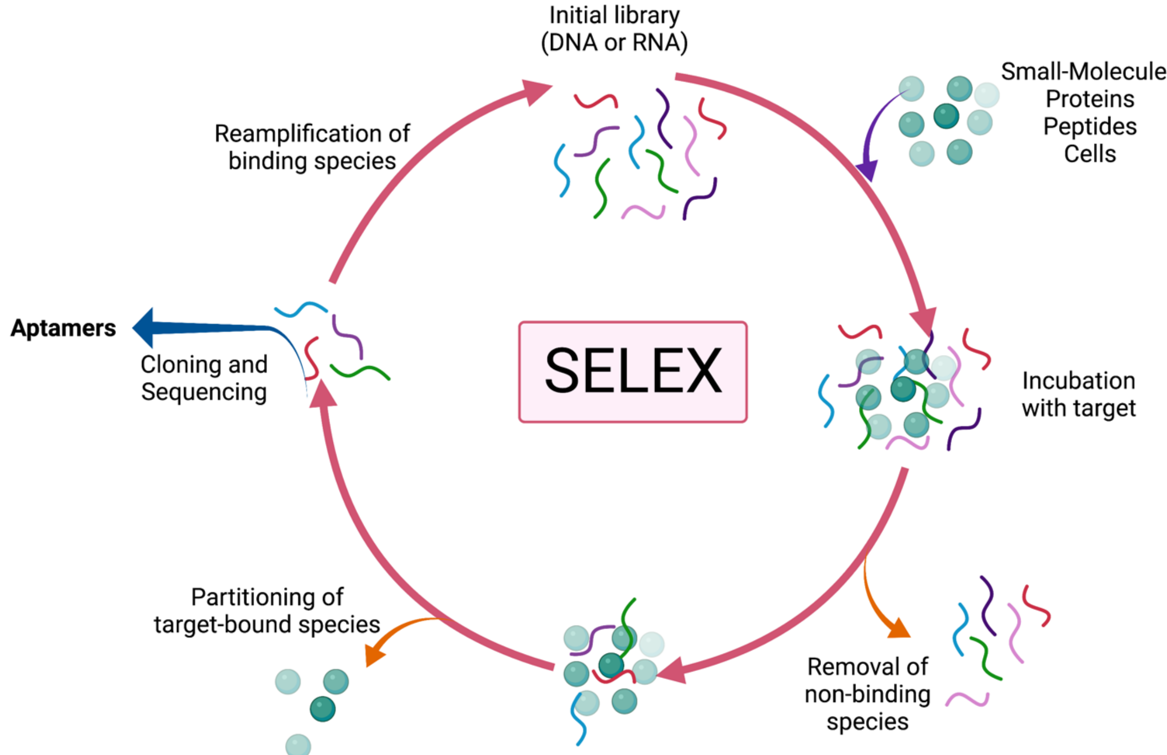


Figure 1.12: Generic SELEX process. We start with a diverse and vast library of ssDNA or ssRNA and incubate with target of interest. After multiple rounds unique binding-sequences are identified.

Aptamer sequences generated by SELEX are typically 60-100 nt in length but can be minimized post-selection to provide significantly shorter sequence lengths, which facilitates their synthesis and use in downstream applications.[95] Due to their facile chemical synthesis and modification, as well as their ability to undergo reversible thermal denaturation, aptamers have found wide use applications where their binding capabilities are used for therapeutics, targeting, or detection of specific analytes.

The SELEX process is outlined in **Figure 1.12.** The general process includes the incubation of a diverse and vast library of single stranded DNA or RNA and incubated with the target of interest (small-molecules, proteins, peptides, cells, etc.). Oftentimes small molecules must be bound to beads beforehand to ensure separation in later steps. After incubation with the target, the removal of non-binding species is performed (via washing), which assures the target-specie complexes remain in the reaction mixture. Further partitioning of the target-bound species allows us to remove the desired DNA or RNA sequences that have affinity to the target. These sequences are them reamplifies and this process can occur over and over again (10-20 rounds). After successful binding rounds, the binding species are cloned and sequenced. This affords useful aptamers that can then be used for detection or sequestration purposes.[96]

Because the nature of the targets, there has been an increase in the types and environments SELEX processes have been developed in. Thinking about the toxins in water, the SELEX processes have to account for the environment surrounding the toxins. So there have been improvements into the study and the implementation of different SELEX processes in lake water.[97] Over the last few years, there has been an increase in the development of aptamers for water toxins. We will discuss some of the newest and most important methods of selection towards small molecules.

Porphyrin. Li and coworkers were able to find a new strategy against this small molecule toxin. In this case they used gold nanoparticles (AuNPs) as the separation matrix and Zinc (II)-Protoporphyrin IX as the target molecule. In this case there was no immobilization step due to the absorption of the DNA to gold. They used N-methyl mesoporphyrin IX to test the progress of the reaction due to its enhancement of fluorescence. They were able to find a truncated aptamer that has

low micromolar K_d, with good fluorescent enhancement. This kind of aptamer serves a dual purpose because it has the potential to be a light-up fluorescent probe.[98]

Gold SELEX. In this type of selection, the need to immobilize the target is no longer necessary because as it binds to the molecules of interest it will detach from the gold nanoparticle (since DNA binds to gold to begin with). Only the molecule-binding DNA complexes will move forward to the next round. As a proof-of-concept, Chatterjee and coworkers were able to develop aptamers against dichlorvos, which then they turned into a colorimetric assay using aptamer-NanoZyme. They developed an aptamer with high affinity (sub micromolar range) and was able to be detected in concentrations as low as 15 μM. They assed practical application by testing this technology in lake water and apple juice.[99]

Base-Modified Aptamer Discovery. Usually, base modification improves the affinity relative to the natural DNA or RNA aptamers. Selection of base-modified aptamers is often quite difficult, for this reason, Gordon and coworkers combined click chemistry strategy with fluorescence activated cell sorting- which measures the affinity and specificity of individual aptamers at a throughput of 10^7 per hour. With this technology they were able to obtain a boronic acid modified aptamer with 1μM affinity for epinephrine.[100]

The SELEX technology keeps improving every day, and now there is an increased number of aptamers selected for a variety of molecules. For this reason, it is important to assume that the use of aptamer for environmental purposes and applications will keep increasing. There are currently numerous aptamers for environmental toxins that have affinity in a variety of mixed matrices.

1.6.2 Detection of Small-Molecule Toxins

Quantification of small molecule toxins in environmental samples is crucial for determining potential exposure levels. Bioanalytical sensors have shown great promise over traditional methods due to their high versatility and field deploy-ability. There are several types of biosensors, which combine a biological occurrence with a physicochemical output. Aptasensors are biosensors that utilize the molecular recognition of aptamers as the biological occurrence. Aptamers are a promising

alternative to antibodies due to high stability, low cost, and little batch to batch variability. Further, aptamers can be easily regenerated while also offering the high affinity recognition like antibodies. Modification of aptamers for attachment to biosensor surfaces or fluorophores is easily obtained without disrupting function. While molecules <1 kDa are challenging targets for antibodies, there are several reports of high affinity aptamers to small molecule toxins. An added advantage of aptamers over other molecular recognition modules is that aptamers can be evolved for toxic targets, making them well suited for environmental monitoring. These aptamers have been further utilized in biosensors due to a *reversible* conformational change upon binding the target of interest. This not only extends the lifetime and useability of the sensor but also allows for real time monitoring. For these reasons, aptamers are harnessed to measure toxin concentration by some readable output, most often electrochemical or optical.[101]

Once aptamers are characterized, they can be incorporated into a bioanalytical sensor. There are several platforms that can be utilized with DNA aptamers. The most promising platforms and implementation strategies for small molecule environmental toxins are:

Label-free Aptasensor for Bisphenol A. Jia and coworkers were able to truncate the known aptamer for BPA (63 nt) to 38 and 12 nt. These aptamers were then used in a label-free colorimetric detection assay based on gold nanoparticles. They were able to obtain lower limits of detection (7.60 pM and 14.41 pM) than the parent DNA. They were able to test it in a variety of media: milk, orange juice, and mineralized water. This technology helps to find this contaminant not only in environmental samples, but also food, which is also a concern of human exposure.[102]

Magnetic-Assisted Fluorescence Probe. Jiang and coworkers were able to use a rapid and sensitive fluorescence assay using 6-carboxy-flurescein labeled aptamer against trichlorfon, glyphosate, and malathion with limits of detection of 72, 88 and 195 ng/L. The aptamer hybridized to magnetic nanoparticles and released upon binding to the molecule, producing a florescence readout. They were able to achieve this in spiked lettuce and carrot samples, therefore showing its broader applicability.[103]

Exonuclease I Electrochemical Aptasensor. Suea-Ngam and coworkers were able to report an ultrasensitive electroanalytical aptasensor for the small-molecule contaminant ochratoxin A. This sensor uses the ability of the DNA aptamer to capture OTA, and silver metallization as a signal enhancer. Exonuclease I then is used to digest the unbound aptamers allowing to detect the small molecule with a limit of detection of 0.7 pg/mL. This method was then compared with UPLC and had less than 5% relative standard deviation.[104]

Electrochemical aptasensors are among the most used for monitoring small molecule environmental toxins. The main principle of these biosensors is to have an aptamer as the recognition agent for a molecule of interest, which upon binding, triggers an electrical change which is propagated through a transducer to a detectable readout. Beyond the advantages that aptamers bring, electrochemical biosensors show great promise for environmental monitoring of small molecule toxins.[105] Advantages include high sensitivity, reproducibility, and stability. One major consideration in electrochemical aptasensor generation that can determine its efficacy is the immobilization of the aptamer to the electrode surface. It is crucial to have efficient immobilization while not restricting the binding of the aptamer to its target. Covalent attachment to electrode surfaces is the most promising as it allows for high loading efficiency and can be easily achieved using modifications of aptamers using various bioconjugation techniques. As it can be seen in the previous examples, attachment to gold surfaces is widely adopted for electrochemical detection. Aptamers can be easily functionalized with a 5' thiol, which allows for conjugation to gold surfaces as a self-assembled monolayer, which can also be directly coupled to a sensor due to its ability to be an electrochemical mediator. This covalent bond is very stable and can be dried down on the surface for long term storage. However, one disadvantage of using gold is the associated cost for on site, disposable detection. Similar to covalent attachment, affinity interactions can be exploited for construction of electrochemical biosensors.[106]

While covalent and affinity interactions are the most used, both of these methods require modification of the aptamer for efficient immobilization. In some cases, selective target recognition

is only accomplished if all regions are bound, such as with the Ochratoxin A aptamer, which has been shown to be negatively affected by different immobilization strategies. Another immobilization technique that circumvents these issues is adsorption.[107] This can be accomplished using graphene oxide, where aptamers can be immobilized through π-π stacking interactions. The major benefit of this strategy is that the aptamers are not restricted, which eliminates the need for both aptamer and target modification, which is a major limitation in small molecule detection. Further, graphene oxide coupled to electrochemical detection led to an increase in sensitivity because it is independent of target size. Beyond this, graphene oxide can be used as a versatile surface for attachment of additional nanomaterials such as nanoparticles, which are often used for improved readout. The last common strategy is through hybridization of the aptamer to a complementary strand immobilized with a previously mentioned technique. While promising, this can lead to high background signal and can affect aptamer binding.[108]

As it can be observed, there is an increase in the amount of small-molecule detection technology using aptamers. Aptamers have proven to be a reliable source of detection- when coupled to a colorimetric or an electrochemical readout. There has been a lot of innovation over the years that prompt this technology to be user-friendly and can be used in point-of-use technologies: paper, electronics, pregnancy-type test kits, etc. It all relies upon the physical characteristics of aptamers themselves, as they are stable and are able to be transported at room temperature. This opens up a wide variety of applications for aptamers, like for example, the removal itself of small-molecule toxins.

1.6.3 Sequestration and Removal of Small-Molecule Toxins

Taking in consideration the number of technologies for the selection and detection of aptamers against small-molecule contaminants and toxins, research has moved towards the use of these functional nucleic acid to perform decontamination itself. Whilst most of the aptamer technologies have been focused on therapeutic applications, aptamers have shown promise in environmental

applications such as the detection and removal of small-molecule toxins and contaminants.[24] To achieve sequestration, the aptamer must be immobilized on a solid support, and scaffolds that have been explored include:

Apta-Decontamination of Cocaine and Diclofenac. Hu and coworkers were able to attach the aptamers cocaine and diclofenac to a column and was able to observe the removal of these contaminants. Attachment with SNBr-sepharose beads was able to show good depletion even after 30 days stored at 4 °C. The removal of pharmaceutical was high at 88-95%. They were also able to explore the kinetics of adsorption.[109]

Aptamer-Assisted Decontamination of Bacteria. In this technology, Song and coworkers were able to remove Escherichia coli with the assistance of three aptamers coupled to TiO_2 particles. The aptamers have affinity to different proteins in the surface of the bacteria. Although efficient, the system does not disinfect the water and it need the usage of UV irradiation after the aptamers sequester the bacteria.[110]

Aptamer-Assisted Decontamination using Liposomes. In this technology the aptamers for three different contaminants (Oxytetracycline, Bisphenol A, and 17-β Estradiol) were inserted into a liposome structure. Then when it comes in contact with the contaminated water, they are able to remove the contaminants and toxins from the water source. The only drawback to this technology is that the aptamers must be in their binding buffer. The capture efficiency is not high when aptamers are used in tap water.[111]

All of these methods, while effective, most of these methods require expensive machinery for fabrication or implementation and they can be susceptible to corrosion or biofouling. Additionally, most of these materials have not been shown to be capable of regeneration, limiting them to a single use.

1.7 Motivation for Study

Taking into consideration what we have discussed thus far, current methods for water purification either require large amounts of electricity or have low efficiency at removing small toxins from water. We propose that using aptamers to remove small toxins from water samples would overcome these challenges. Specifically, we propose the conjugation of aptamers to an ultrafiltration membrane that could then be incorporated into an existing water filtration technology. This approach would avoid the use of electricity and high pressure, making the use of personal water purification possible in geographically remote locations. This thesis discusses the preparation, casting, grafting, aptamer functionalization, and characterization of the polymeric membrane. While this investigation focuses only on small-molecule contaminants, cyanotoxins, and bacteria, successful removal of toxin molecules will set the precedent for using aptamers in membrane materials for water purification. Additionally, aptamers can be selected to remove target small molecules from water depending on local community needs.

1.8 Summary and Objectives of this Dissertation

The objective of this work is to develop an aptamer-based filtration system for the removal of small-molecule contaminant and toxins from contaminated water by combining membrane engineering and chemical biology. One of the goals of this project is to achieve the desired surface characteristics without loss of the advantageous properties of ultrafiltration PES membranes. Poly-methacrylic acid chains (PMAA)-grafted membrane of PES is anticipated to possess favorable selectivity-permeability characteristics due to the dual hydrophobic/hydrophilic nature of the membrane surface. Moreover, PES has a glass transition temperature of 230 °C, meaning that it will have sufficient thermal stability when aptamers are to be regenerated in water samples at elevated temperatures. The PMAA added to the membrane surface and pores will serve as a point-of-attachment for aptamer conjugation while conferring some degree of hydrophilicity to the

membrane. Within the various next generation membrane systems, we anticipate that the use of aptamer-functionalized membranes will be of high value. This is because we will be able to purify fresh water and remove low-concentration contaminants in high backgrounds of potable constituents at a lower cost.

Once the aptamer-functionalized membranes are obtained, we will measure small-molecule removal efficiency by filtering water contaminated with small-molecules at different concentrations. We will determine the amount of small-molecules that was removed by the membrane by the difference in concentration between the contaminated water and the permeate. Membrane performance will be measured by filtering contaminated water through the membrane multiple times until the loading of toxins is at its maximum. We will also explore the ability of the aptamers to be regenerated by eluting away the bound toxin. As it was stated before, aptamers denature at high temperatures, but regain activity when cooled down. Hence, we will explore the regeneration by washing through with varying temperatures of water and then track membrane performance after regeneration.

When we have an optimized system for the removal of small-molecule toxins, we set forth into exploring other applications of the aptamer-functionalized membranes. An exciting avenue that we explore is the degradation of the molecules that are being depleted by our aptamer-functionalized membrane. We envisioned that if we can attach an enzyme capable of degrading contaminants, then we can have a membrane that is regenerable on its own and it does not need further treatment to release the small molecule (often achieved by changes in pH or temperature). We were able to attach the enzyme Laccase to our membranes and use BPA as our model system. Laccase is able to degrade BPA into different degradation products that are no longer harmful for the human body. With this system we are able to achieve a dual-activity membrane that not only removes the small-molecule contaminant but also degrades it. With the appropriate design of enzyme and aptamer, this technology can be used to deplete and degrade harmful molecules from water. Another exciting technique we are currently exploring is introducing a more

delicate structural design with a highly-ordered single layer of deposition of silica nanoparticles. Silica nanoparticles are known for their lack of cytotoxicity, pH resistance, and are chemically amenable and easy to synthesize. We examine the attachment and functionalization strategy of the nanoparticles. We are preparing amino-modified silica particles that will be able to covalently bind onto the membrane. Due to the single-layer compacting design, the thickness of the membrane can be reduced to low micron levels.

Due to the imminence of fresh-water scarcity, it has become critical to ensure we develop efficient methods that can sequester and remove micro- and macro- molecules from our water supplies. We set forth to achieve this by leveraging the key properties of ultrafiltration membranes and combining them with aptamers- a powerful biological tool. Our prudent method utilizes green chemistry and reagents, further enhancing sustainability and does not require large amounts of electricity or pressure, thus promoting a “budget-friendly” alternative. Furthermore, we envisage our water purification strategy will be able to rapidly sequester multiple small-molecule components, and the scope can be eventually expanded to recover small molecules.

1.9 References

1. Niccolucci, V.; Botto, S.; Rugani, B.; Nicolardi, V.; Bastianoni, S.; Gaggi, C., The real water consumption behind drinking water: The case of Italy. *Journal of Environmental Management* **2011,** *92* (10), 2611-2618.

2. Daud, M. K.; Nafees, M.; Ali, S.; Rizwan, M.; Bajwa, R. A.; Shakoor, M. B.; Arshad, M. U.; Chatha, S. A. S.; Deeba, F.; Murad, W.; Malook, I.; Zhu, S. J., Drinking Water Quality Status and Contamination in Pakistan. *BioMed Research International* **2017,** *2017*, 7908183.

3. Díaz-Caravantes, R. E.; Wilder, M., Water, Cities and Peri-urban Communities: Geographies of Power in the Context of Drought in Northwest Mexico. *Water Alternatives* **2014,** *7* (3), 499-517.

4. Zhang, X.; Chen, N.; Sheng, H.; Ip, C.; Yang, L.; Chen, Y.; Sang, Z.; Tadesse, T.; Lim, T. P. Y.; Rajabifard, A.; Bueti, C.; Zeng, L.; Wardlow, B.; Wang, S.; Tang, S.; Xiong, Z.; Li, D.; Niyogi, D., Urban drought challenge to 2030 sustainable development goals. *Science of The Total Environment* **2019,** *693*, 133536.

5. Sharma, S.; Bhattacharya, A., Drinking water contamination and treatment techniques. *Applied Water Science* **2017,** *7* (3), 1043-1067.

6. Bertoldi, C.; Lara, L. Z.; Mizushima, F. A. d. L.; Martins, F. C. G.; Battisti, M. A.; Hinrichs, R.; Fernandes, A. N., First evidence of microplastic contamination in the freshwater of Lake Guaíba, Porto Alegre, Brazil. *Science of The Total Environment* **2021,** *759*, 143503.

7. Schiedek, D.; Sundelin, B.; Readman, J. W.; Macdonald, R. W., Interactions between climate change and contaminants. *Marine Pollution Bulletin* **2007,** *54* (12), 1845-1856.

8. Daly, G. L.; Wania, F., Organic Contaminants in Mountains. *Environmental Science & Technology* **2005,** *39* (2), 385-398.

9. Whelton, A. J.; McMillan, L.; Connell, M.; Kelley, K. M.; Gill, J. P.; White, K. D.; Gupta, R.; Dey, R.; Novy, C., Residential Tap Water Contamination Following the Freedom Industries

Chemical Spill: Perceptions, Water Quality, and Health Impacts. *Environmental Science & Technology* **2015,** *49* (2), 813-823.

10. Kashem, M. D. A.; Singh, B. R., Heavy Metal Contamination of Soil and Vegetation in the Vicinity of Industries in Bangladesh. *Water, Air, and Soil Pollution* **1999,** *115* (1), 347-361.

11. Mason, S. A.; Welch, V. G.; Neratko, J., Synthetic Polymer Contamination in Bottled Water. *Frontiers in Chemistry* **2018,** *6* (407).

12. Malato, S.; Fernández-Ibáñez, P.; Maldonado, M. I.; Blanco, J.; Gernjak, W., Decontamination and disinfection of water by solar photocatalysis: Recent overview and trends. *Catalysis Today* **2009,** *147* (1), 1-59.

13. Szabo, J.; Minamyer, S., Decontamination of biological agents from drinking water infrastructure: A literature review and summary. *Environment International* **2014,** *72*, 124-128.

14. Samantaray, P. K.; Kumar, S.; Bose, S., 'Polycation' modified PVDF based antibacterial and antifouling membranes and 'point-of-use supports' for sustainable and effective water decontamination. *Journal of Water Process Engineering* **2020,** *38*, 101536.

15. Agrawal, V. K.; Bhalwar, R., Household Water Purification: Low-Cost Interventions. *Medical Journal Armed Forces India* **2009,** *65* (3), 260-263.

16. Dongre, R. S.; Sadasivuni, K. K.; Deshmukh, K.; Mehta, A.; Basu, S.; Meshram, J. S.; Al-Maadeed, M. A. A.; Karim, A., Natural polymer based composite membranes for water purification: a review. *Polymer-Plastics Technology and Materials* **2019,** *58* (12), 1295-1310.

17. Ali, M. I.; Summers, E. K.; Arafat, H. A.; V, J. H. L., Effects of membrane properties on water production cost in small scale membrane distillation systems. *Desalination* **2012,** *306*, 60-71.

18. Miyoshi, T.; Yuasa, K.; Ishigami, T.; Rajabzadeh, S.; Kamio, E.; Ohmukai, Y.; Saeki, D.; Ni, J.; Matsuyama, H., Effect of membrane polymeric materials on relationship between surface pore size and membrane fouling in membrane bioreactors. *Applied Surface Science* **2015,** *330*, 351-357.

19. Kim, S.; Chu, K. H.; Al-Hamadani, Y. A. J.; Park, C. M.; Jang, M.; Kim, D.-H.; Yu, M.; Heo, J.; Yoon, Y., Removal of contaminants of emerging concern by membranes in water and wastewater: A review. *Chemical Engineering Journal* **2018,** *335*, 896-914.

20. Xu, W.; He, W.; Du, Z.; Zhu, L.; Huang, K.; Lu, Y.; Luo, Y., Functional Nucleic Acid Nanomaterials: Development, Properties, and Applications. *Angewandte Chemie International Edition* **2021,** *60* (13), 6890-6918.

21. Adachi, T.; Nakamura, Y., Aptamers: A Review of Their Chemical Properties and Modifications for Therapeutic Application. *Molecules* **2019,** *24* (23).

22. Liang, G.; Man, Y.; Li, A.; Jin, X.; Liu, X.; Pan, L., DNAzyme-based biosensor for detection of lead ion: A review. *Microchemical Journal* **2017,** *131*, 145-153.

23. Ding, F.; Gao, Y.; He, X., Recent progresses in biomedical applications of aptamer-functionalized systems. *Bioorganic & Medicinal Chemistry Letters* **2017,** *27* (18), 4256-4269.

24. Bilibana, M. P.; Citartan, M.; Yeoh, T. S.; Rozhdestvensky, T. S.; Tang, T.-H., Aptamers as the Agent in Decontamination Assays (Apta-Decontamination Assays): From the Environment to the Potential Application<i> In Vivo</i>. *Journal of Nucleic Acids* **2017,** *2017*, 3712070.

25. Albert, J. S.; Destouni, G.; Duke-Sylvester, S. M.; Magurran, A. E.; Oberdorff, T.; Reis, R. E.; Winemiller, K. O.; Ripple, W. J., Scientists' warning to humanity on the freshwater biodiversity crisis. *Ambio* **2021,** *50* (1), 85-94.

26. Kalinkat, G.; Jähnig, S. C.; Jeschke, J. M., Exceptional body size–extinction risk relations shed new light on the freshwater biodiversity crisis. *Proceedings of the National Academy of Sciences* **2017,** *114* (48), E10263.

27. Borgwardt, F.; Robinson, L.; Trauner, D.; Teixeira, H.; Nogueira, A. J. A.; Lillebø, A. I.; Piet, G.; Kuemmerlen, M.; O'Higgins, T.; McDonald, H.; Arevalo-Torres, J.; Barbosa, A. L.; Iglesias-Campos, A.; Hein, T.; Culhane, F., Exploring variability in environmental impact risk from human activities across aquatic ecosystems. *Science of The Total Environment* **2019,** *652*, 1396-1408.

28. Köhler, H., Individual metering and debiting (IMD) in Sweden: A qualitative long-term follow-up study of householders' water-use routines. *Energy Policy* **2017,** *108*, 344-354.

29. Bierkens, M. F. P.; Wada, Y., Non-renewable groundwater use and groundwater depletion: a review. *Environmental Research Letters* **2019,** *14* (6), 063002.

30. Jia, X.; O'Connor, D.; Hou, D.; Jin, Y.; Li, G.; Zheng, C.; Ok, Y. S.; Tsang, D. C. W.; Luo, J., Groundwater depletion and contamination: Spatial distribution of groundwater resources sustainability in China. *Science of The Total Environment* **2019,** *672*, 551-562.

31. Dalin, C.; Wada, Y.; Kastner, T.; Puma, M. J., Groundwater depletion embedded in international food trade. *Nature* **2017,** *543* (7647), 700-704.

32. Tuninetti, M.; Tamea, S.; Dalin, C., Water Debt Indicator Reveals Where Agricultural Water Use Exceeds Sustainable Levels. *Water Resources Research* **2019,** *55* (3), 2464-2477.

33. Boretti, A.; Rosa, L., Reassessing the projections of the World Water Development Report. *npj Clean Water* **2019,** *2* (1), 15.

34. Qu, S.; Liang, S.; Konar, M.; Zhu, Z.; Chiu, A. S. F.; Jia, X.; Xu, M., Virtual Water Scarcity Risk to the Global Trade System. *Environmental Science & Technology* **2018,** *52* (2), 673-683.

35. Vanham, D.; Hoekstra, A. Y.; Bidoglio, G., Potential water saving through changes in European diets. *Environment International* **2013,** *61*, 45-56.

36. Jalava, M.; Kummu, M.; Porkka, M.; Siebert, S.; Varis, O., Diet change—a solution to reduce water use? *Environmental Research Letters* **2014,** *9* (7), 074016.

37. Arora, N. K.; Mishra, I., United Nations Sustainable Development Goals 2030 and environmental sustainability: race against time. *Environmental Sustainability* **2019,** *2* (4), 339-342.

38. Gleick, P. H., WATER IN CRISIS: PATHS TO SUSTAINABLE WATER USE. *Ecological Applications* **1998,** *8* (3), 571-579.

39. Patra, D. K.; Pradhan, C.; Patra, H. K., Toxic metal decontamination by phytoremediation approach: Concept, challenges, opportunities and future perspectives. *Environmental Technology & Innovation* **2020,** *18*, 100672.

40. Zhang, X.; Gu, P.; Liu, Y., Decontamination of radioactive wastewater: State of the art and challenges forward. *Chemosphere* **2019,** *215*, 543-553.

41. Warner, J. X.; Dickerson, R. R.; Wei, Z.; Strow, L. L.; Wang, Y.; Liang, Q., Increased atmospheric ammonia over the world's major agricultural areas detected from space. *Geophysical Research Letters* **2017,** *44* (6), 2875-2884.

42. Abu Hasan, H.; Muhammad, M. H.; Ismail, N. I., A review of biological drinking water treatment technologies for contaminants removal from polluted water resources. *Journal of Water Process Engineering* **2020,** *33*, 101035.

43. Manyatshe, A.; Fosso-Kankeu, E.; van der Berg, D.; Lemmer, N.; Waanders, F.; Tutu, H., Dispersion of inorganic contaminants in surface water in the vicinity of Potchefstroom. *Physics and Chemistry of the Earth, Parts A/B/C* **2017,** *100*, 86-93.

44. Qi, C.; Liu, X.; Li, Y.; Lin, C.; Ma, J.; Li, X.; Zhang, H., Enhanced degradation of organic contaminants in water by peroxydisulfate coupled with bisulfite. *Journal of Hazardous Materials* **2017,** *328*, 98-107.

45. Zulkifli, S. N.; Rahim, H. A.; Lau, W.-J., Detection of contaminants in water supply: A review on state-of-the-art monitoring technologies and their applications. *Sensors and Actuators B: Chemical* **2018,** *255*, 2657-2689.

46. Sharma, V. P.; Sharma, P., Chapter 5 - Environmental Contaminants: Treatment, Threats, Toxicity, and Tools for Sustainability. In *Wastewater Treatment*, Shah, M. P.; Sarkar, A.; Mandal, S., Eds. Elsevier: 2021; pp 93-102.

47. Shah, A. I.; Din Dar, M. U.; Bhat, R. A.; Singh, J. P.; Singh, K.; Bhat, S. A., Prospectives and challenges of wastewater treatment technologies to combat contaminants of emerging concerns. *Ecological Engineering* **2020,** *152*, 105882.

48. Ochowiak, M.; Matuszak, M.; Włodarczak, S.; Ancukiewicz, M.; Krupińska, A., The modified swirl sedimentation tanks for water purification. *Journal of Environmental Management* **2017,** *189*, 22-28.

49. Teixeira, M. R.; Rosa, M. J., Comparing dissolved air flotation and conventional sedimentation to remove cyanobacterial cells of Microcystis aeruginosa: Part II. The effect of water background organics. *Separation and Purification Technology* **2007,** *53* (1), 126-134.

50. Tarpagkou, R.; Pantokratoras, A., The influence of lamellar settler in sedimentation tanks for potable water treatment — A computational fluid dynamic study. *Powder Technology* **2014,** *268*, 139-149.

51. Fang, H.-F.; Wang, C.-F.; Lin, C.-K., Improving distillation method and device of tritiated water analysis for ultra high decontamination efficiency. *Journal of Environmental Radioactivity* **2015,** *150*, 62-67.

52. Garrigues, S.; Armenta, S.; Guardia, M. d. l., Green strategies for decontamination of analytical wastes. *TrAC Trends in Analytical Chemistry* **2010,** *29* (7), 592-601.

53. Kurniawan, T. A.; Chan, G. Y. S.; Lo, W.-H.; Babel, S., Physico–chemical treatment techniques for wastewater laden with heavy metals. *Chemical Engineering Journal* **2006,** *118* (1), 83-98.

54. Alimi, F.; Tlili, M. M.; Amor, M. B.; Maurin, G.; Gabrielli, C., Effect of magnetic water treatment on calcium carbonate precipitation: Influence of the pipe material. *Chemical Engineering and Processing: Process Intensification* **2009,** *48* (8), 1327-1332.

55. Fathi, A.; Mohamed, T.; Claude, G.; Maurin, G.; Mohamed, B. A., Effect of a magnetic water treatment on homogeneous and heterogeneous precipitation of calcium carbonate. *Water Research* **2006,** *40* (10), 1941-1950.

56. Sher, F.; Malik, A.; Liu, H., Industrial polymer effluent treatment by chemical coagulation and flocculation. *Journal of Environmental Chemical Engineering* **2013,** *1* (4), 684-689.

57. Saritha, V.; Srinivas, N.; Srikanth Vuppala, N. V., Analysis and optimization of coagulation and flocculation process. *Applied Water Science* **2017,** *7* (1), 451-460.

58. Dotto, J.; Fagundes-Klen, M. R.; Veit, M. T.; Palácio, S. M.; Bergamasco, R., Performance of different coagulants in the coagulation/flocculation process of textile wastewater. *Journal of Cleaner Production* **2019,** *208*, 656-665.

59. Ali, I., New Generation Adsorbents for Water Treatment. *Chemical Reviews* **2012,** *112* (10), 5073-5091.

60. Bhatnagar, A.; Hogland, W.; Marques, M.; Sillanpää, M., An overview of the modification methods of activated carbon for its water treatment applications. *Chemical Engineering Journal* **2013,** *219*, 499-511.

61. Hao, J.; Han, M.-J.; Meng, X., Preparation and evaluation of thiol-functionalized activated alumina for arsenite removal from water. *Journal of Hazardous Materials* **2009,** *167* (1), 1215-1221.

62. Yang, R.; Li, H.; Huang, M.; Yang, H.; Li, A., A review on chitosan-based flocculants and their applications in water treatment. *Water Research* **2016,** *95*, 59-89.

63. De Velasco-Maldonado, P. S.; Hernández-Montoya, V.; Montes-Morán, M. A.; Vázquez, N. A.-R.; Pérez-Cruz, M. A., Surface modification of a natural zeolite by treatment with cold oxygen plasma: Characterization and application in water treatment. *Applied Surface Science* **2018,** *434*, 1193-1199.

64. Awasthi, A.; Jadhao, P.; Kumari, K., Clay nano-adsorbent: structures, applications and mechanism for water treatment. *SN Applied Sciences* **2019,** *1* (9), 1076.

65. Cappelli, G.; Riccardi, M.; Perrone, S.; Bondi, M.; Ligabue, G.; Albertazzi, A., Water treatment and monitor disinfection. *Hemodialysis International* **2006,** *10* (s1), S13-S18.

66. Feng, C.; Suzuki, K.; Zhao, S.; Sugiura, N.; Shimada, S.; Maekawa, T., Water disinfection by electrochemical treatment. *Bioresource Technology* **2004,** *94* (1), 21-25.

67. Kim, J.; Chung, Y.; Shin, D.; Kim, M.; Lee, Y.; Lim, Y.; Lee, D., Chlorination by-products in surface water treatment process. *Desalination* **2003,** *151* (1), 1-9.

68. Backer, H.; Hollowell, J., Use of iodine for water disinfection: iodine toxicity and maximum recommended dose. *Environmental Health Perspectives* **2000,** *108* (8), 679-684.

69. Zularisam, A. W.; Ismail, A. F.; Salim, R., Behaviours of natural organic matter in membrane filtration for surface water treatment — a review. *Desalination* **2006,** *194* (1), 211-231.

70. Babu, R.; Chaudhuri, M., Home water treatment by direct filtration with natural coagulant. *Journal of Water and Health* **2005,** *3* (1), 27-30.

71. Lee, A.; Elam, J. W.; Darling, S. B., Membrane materials for water purification: design, development, and application. *Environmental Science: Water Research & Technology* **2016,** *2* (1), 17-42.

72. Fane, A. G.; Wang, R.; Hu, M. X., Synthetic Membranes for Water Purification: Status and Future. *Angewandte Chemie International Edition* **2015,** *54* (11), 3368-3386.

73. Pusch, W.; Walch, A., Synthetic Membranes—Preparation, Structure, and Application. *Angewandte Chemie International Edition in English* **1982,** *21* (9), 660-685.

74. Li, Y.; Liu, Y.; Yang, Y.; Li, Y.-Q.; Zhao, M.; Li, W.; Qu, Y., Efficient isotropic water desalination in anisotropic lamellar nano-channels formed by layered black phosphorus membrane. *Desalination* **2021,** *504*, 114962.

75. Zhao, X.; Tong, Z.; Liu, X.; Wang, J.; Zhang, B., Facile Preparation of Polyamide–Graphene Oxide Composite Membranes for Upgrading Pervaporation Desalination Performances of Hypersaline Solutions. *Industrial & Engineering Chemistry Research* **2020,** *59* (26), 12232-12238.

76. Sionkowska, A., Current research on the blends of natural and synthetic polymers as new biomaterials: Review. *Progress in Polymer Science* **2011,** *36* (9), 1254-1276.

77. Thakur, V. K.; Voicu, S. I., Recent advances in cellulose and chitosan based membranes for water purification: A concise review. *Carbohydrate Polymers* **2016,** *146*, 148-165.

78. Miller, D. J.; Dreyer, D. R.; Bielawski, C. W.; Paul, D. R.; Freeman, B. D., Surface Modification of Water Purification Membranes. *Angewandte Chemie International Edition* **2017,** *56* (17), 4662-4711.

79. Yang, Z.; Zhou, Y.; Feng, Z.; Rui, X.; Zhang, T.; Zhang, Z., A Review on Reverse Osmosis and Nanofiltration Membranes for Water Purification. *Polymers* **2019,** *11* (8), 1252.

80. Jun, B.-M.; Al-Hamadani, Y. A. J.; Son, A.; Park, C. M.; Jang, M.; Jang, A.; Kim, N. C.; Yoon, Y., Applications of metal-organic framework based membranes in water purification: A review. *Separation and Purification Technology* **2020,** *247*, 116947.

81. Mottern, M. L.; Shqau, K.; Shi, J. Y.; Yu, D.; Verweij, H., Thin supported inorganic membranes for energy-related gas and water purification. *International Journal of Hydrogen Energy* **2007,** *32* (16), 3713-3723.

82. Lewis, S. R.; Datta, S.; Gui, M.; Coker, E. L.; Huggins, F. E.; Daunert, S.; Bachas, L.; Bhattacharyya, D., Reactive nanostructured membranes for water purification. *Proceedings of the National Academy of Sciences* **2011,** *108* (21), 8577.

83. Qadir, D.; Mukhtar, H.; Keong, L. K., Mixed Matrix Membranes for Water Purification Applications. *Separation & Purification Reviews* **2017,** *46* (1), 62-80.

84. Schäfer, A. I.; Fane, A. G.; Waite, T. D., Fouling effects on rejection in the membrane filtration of natural waters. *Desalination* **2000,** *131* (1), 215-224.

85. Rautenbach, R.; Linn, T.; Eilers, L., Treatment of severely contaminated waste water by a combination of RO, high-pressure RO and NF — potential and limits of the process. *Journal of Membrane Science* **2000,** *174* (2), 231-241.

86. Fiksdal, L.; Leiknes, T., The effect of coagulation with MF/UF membrane filtration for the removal of virus in drinking water. *Journal of Membrane Science* **2006,** *279* (1), 364-371.

87. Loeb, S. K.; Alvarez, P. J. J.; Brame, J. A.; Cates, E. L.; Choi, W.; Crittenden, J.; Dionysiou, D. D.; Li, Q.; Li-Puma, G.; Quan, X.; Sedlak, D. L.; David Waite, T.; Westerhoff, P.; Kim, J.-H.,

The Technology Horizon for Photocatalytic Water Treatment: Sunrise or Sunset? *Environmental Science & Technology* **2019,** *53* (6), 2937-2947.

88. Patil, R.; Ahmad, D.; Balkundae, P.; Kausley, S.; Malhotra, C., Development of low cost point-of-use (POU) interventions for instant decontamination of drinking water in developing countries. *Journal of Water Process Engineering* **2020,** *37*, 101435.

89. Ng Carla, A.; von Goetz, N., The Global Food System as a Transport Pathway for Hazardous Chemicals: The Missing Link between Emissions and Exposure. *Environmental Health Perspectives* **2017,** *125* (1), 1-7.

90. Ai, Y.; Lee, S.; Lee, J., Drinking water treatment residuals from cyanobacteria bloom-affected areas: Investigation of potential impact on agricultural land application. *Science of The Total Environment* **2020,** *706*, 135756.

91. Halsch, C. A.; Code, A.; Hoyle, S. M.; Fordyce, J. A.; Baert, N.; Forister, M. L., Pesticide Contamination of Milkweeds Across the Agricultural, Urban, and Open Spaces of Low-Elevation Northern California. *Frontiers in Ecology and Evolution* **2020,** *8* (162).

92. Massey, I. Y.; Yang, F.; Ding, Z.; Yang, S.; Guo, J.; Tezi, C.; Al-Osman, M.; Kamegni, R. B.; Zeng, W., Exposure routes and health effects of microcystins on animals and humans: A mini-review. *Toxicon* **2018,** *151*, 156-162.

93. Mehlhorn, A.; Rahimi, P.; Joseph, Y., Aptamer-Based Biosensors for Antibiotic Detection: A Review. *Biosensors* **2018,** *8* (2).

94. Debiais, M.; Lelievre, A.; Smietana, M.; Müller, S., Splitting aptamers and nucleic acid enzymes for the development of advanced biosensors. *Nucleic Acids Research* **2020,** *48* (7), 3400-3422.

95. Kaur, H., Recent developments in cell-SELEX technology for aptamer selection. *Biochimica et Biophysica Acta (BBA) - General Subjects* **2018,** *1862* (10), 2323-2329.

96. Zhuo, Z.; Yu, Y.; Wang, M.; Li, J.; Zhang, Z.; Liu, J.; Wu, X.; Lu, A.; Zhang, G.; Zhang, B., Recent Advances in SELEX Technology and Aptamer Applications in Biomedicine. *International journal of molecular sciences* **2017,** *18* (10), 2142.

97. Wu, Y.; Belmonte, I.; Sykes, K. S.; Xiao, Y.; White, R. J., Perspective on the Future Role of Aptamers in Analytical Chemistry. *Analytical Chemistry* **2019,** *91* (24), 15335-15344.

98. Li, W.; Luo, Y.; Gao, T.; Yang, L.; Wang, J.; Pei, R., In Vitro Selection of DNA Aptamers for a Small-Molecule Porphyrin by Gold Nanoparticle-Based SELEX. *Journal of Molecular Evolution* **2019,** *87* (7), 231-239.

99. Chatterjee, B.; Kalyani, N.; Anand, A.; Khan, E.; Das, S.; Bansal, V.; Kumar, A.; Sharma, T. K., GOLD SELEX: a novel SELEX approach for the development of high-affinity aptamers against small molecules without residual activity. *Microchimica Acta* **2020,** *187* (11), 618.

100. Gordon, C. K. L.; Wu, D.; Pusuluri, A.; Feagin, T. A.; Csordas, A. T.; Eisenstein, M. S.; Hawker, C. J.; Niu, J.; Soh, H. T., Click-Particle Display for Base-Modified Aptamer Discovery. *ACS Chemical Biology* **2019,** *14* (12), 2652-2662.

101. Ruscito, A.; DeRosa, M. C., Small-Molecule Binding Aptamers: Selection Strategies, Characterization, and Applications. *Frontiers in Chemistry* **2016,** *4* (14).

102. Jia, M.; Sha, J.; Li, Z.; Wang, W.; Zhang, H., High affinity truncated aptamers for ultra-sensitive colorimetric detection of bisphenol A with label-free aptasensor. *Food Chemistry* **2020,** *317*, 126459.

103. Jiang, M.; Chen, C.; He, J.; Zhang, H.; Xu, Z., Fluorescence assay for three organophosphorus pesticides in agricultural products based on Magnetic-Assisted fluorescence labeling aptamer probe. *Food Chemistry* **2020,** *307*, 125534.

104. Suea-Ngam, A.; Howes, P. D.; Stanley, C. E.; deMello, A. J., An Exonuclease I-Assisted Silver-Metallized Electrochemical Aptasensor for Ochratoxin A Detection. *ACS Sensors* **2019,** *4* (6), 1560-1568.

105. Sassolas, A.; Blum, L. J.; Leca-Bouvier, B. D., Electrochemical Aptasensors. *Electroanalysis* **2009,** *21* (11), 1237-1250.

106. Xu, Y.; Cheng, G.; He, P.; Fang, Y., A Review: Electrochemical Aptasensors with Various Detection Strategies. *Electroanalysis* **2009,** *21* (11), 1251-1259.

107. Rapini, R.; Marrazza, G., Electrochemical aptasensors for contaminants detection in food and environment: Recent advances. *Bioelectrochemistry* **2017,** *118*, 47-61.

108. Mishra, G. K.; Sharma, V.; Mishra, R. K., Electrochemical Aptasensors for Food and Environmental Safeguarding: A Review. *Biosensors* **2018,** *8* (2), 28.

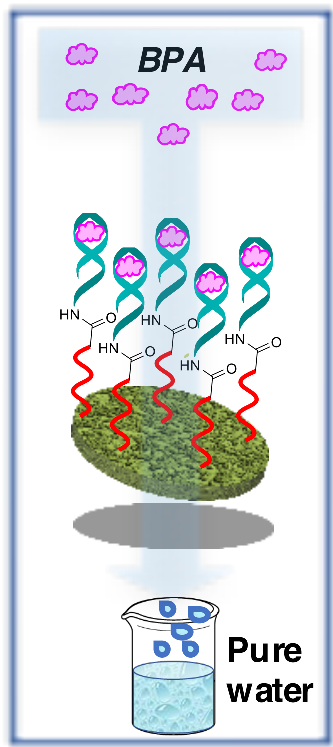
109. Hu, X.; Mu, L.; Zhou, Q.; Wen, J.; Pawliszyn, J., ssDNA Aptamer-Based Column for Simultaneous Removal of Nanogram Per Liter Level of Illicit and Analgesic Pharmaceuticals in Drinking Water. *Environmental Science & Technology* **2011,** *45* (11), 4890-4895.

110. Song, M. Y.; Jurng, J.; Park, Y.-K.; Kim, B. C., An aptamer cocktail-functionalized photocatalyst with enhanced antibacterial efficiency towards target bacteria. *Journal of Hazardous Materials* **2016,** *318*, 247-254.

111. Kim, Y. S.; Niazi, J. H.; Chae, Y. J.; Ko, U. R.; Gu, M. B., Aptamers-in-liposomes for selective and multiplexed capture of small organic compounds. *Macromol Rapid Commun* **2011,** *32* (15), 1169-73.

Chapter 2

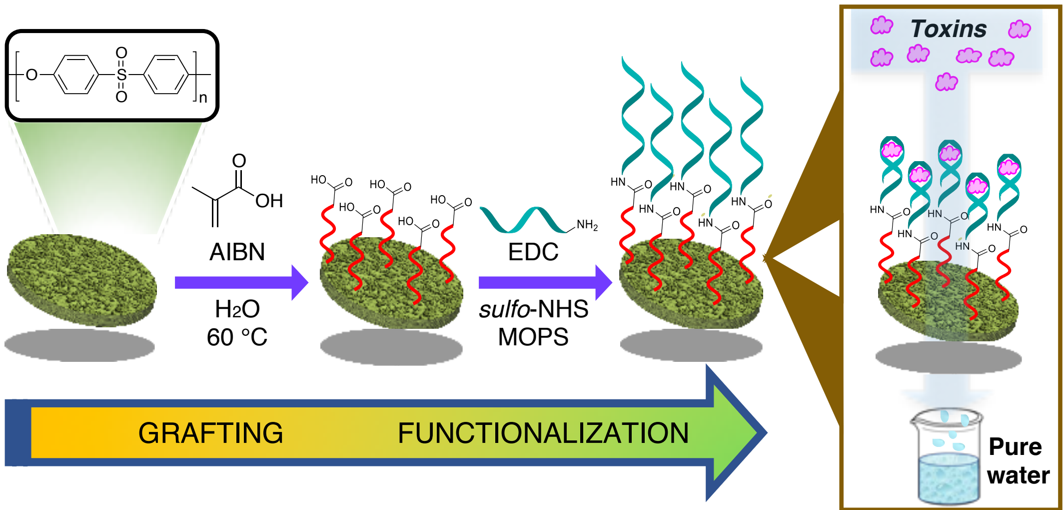
Sequestration of Bisphenol A by the Aptamer-Functionalized Membrane



In this chapter, we prepare an aptamer-functionalized membrane and investigate its properties and functionality in water.

2.1 Abstract

Sequestration of small molecules from aqueous solutions poses a significant, yet important challenge in environmental science and human health. Current methods focus on broadly sequestering all small molecules but are unable to address specific small molecules of interest. Additionally, these procedures require large amounts of resources such as electricity and pressure. We propose to address this challenge through the use of DNA aptamer-functionalized ultrafiltration membranes. To demonstrate this approach, we developed an ap-tamer-functionalized membrane that sequesters and removes the small-molecule contaminant bisphenol A (BPA) from water. We show that BPA can be depleted and that the membranes can be regenerated for multiple uses, which can allow for recovery of the small molecule when desired. Aptamers can be selected for a wide variety of target small molecules, making this approach highly generalizable beyond our initial demonstration. Together, this research offers a promising solution to improving the efficacy of small molecule removal and recovery from aqueous matrices.



2.2 Introduction

Aqueous environments are populated with a diverse array of small molecules, many of which can be either hazardous or beneficial to human health. Thus, there is a significant interest in sequestering these molecules from the aqueous environment, as this allows depletion of harmful analytes such as toxins or contaminants and recovery of valuable analytes such as natural products. Methods including coagulation, flocculation, sedimentation, and photon-based inactivation are capable of removing these contaminants from water. However, these technologies generally require the consumption of additional chemicals and energy, making them difficult to implement beyond industrial settings. Moreover, these approaches can only target small molecules in general and not specific analytes. Therefore, they can only be used for depletion of small molecules, and not for their recovery.

Synthetic membranes offer a promising alternative solution, owing to their facile preparation, ease of use, and minimal resource consumption. Membranes are broadly classified by pore size and internal structure, and ultrafiltration membranes having pore sizes in the high nm to low μm range are widely used for removal of large molecular weight contaminants such as bacteria, parasites, and particulates. However, the larger pore sizes of ultrafiltration membranes make them ineffective at separating small molecules from aqueous solutions. Small molecules can be effectively removed using membranes having smaller pore sizes, however this also increases production cost and the resources needed for use. And, similar to chemical separation methods, relying on pore size for separation only enables sequestration according to molecular size and does not enable the depletion or recovery of specific small-molecule analytes.

We hypothesized that this challenge could be addressed by conjugating small-molecule binding aptamers to ultrafiltration membranes, enabling the sequestration of specific small-molecule analytes while maintaining high ease of use. Aptamers are single-stranded nucleic acids that are capable of binding to a target molecule with high affinity and specificity. We recognized that DNA

aptamers are exceptionally well-suited for use as sequestration agents in the context of ultrafiltration membranes, given these characteristics and their specific ability to be reversibly denatured in response to thermal or chemical stimuli. This can enable the surface of the membrane to be regenerated multiple times, greatly extending its useful lifetime and enabling recovery of small molecule analytes of value.

The ability of aptamers to deplete small molecules from water has been previously investigated using aptamer-functionalized beads that can be packed as a sorbent in “aptamer columns” to remove contaminants such as cocaine, diclofenac, and ochratoxin A from water. However, these approaches require complex preparation techniques, have low flow rates, and lack the ability to simultaneously remove larger contaminants when desired.

We chose BPA as an initial model system, as a well-characterized DNA aptamer is available for this target and BPA is a prevalent contaminant in groundwater and surface water. Techniques for BPA removal do exist but rely upon complicated preparation methods or energy-intensive processes, and often require pre- or post-treatment of the water sample. Here we show that functionalization of ultrafiltration membranes with DNA aptamers enables depletion of BPA, and the membranes can be regenerated for repeated use. Considering the broad range of toxins and contaminants for which DNA aptamers can be generated, we anticipate that this will provide a generalizable and customizable approach to the depletion and recovery of small molecules. The research reported here is the first to demonstrate that the ultrafiltration membranes that are very commonly used for removal of pathogenic microorganisms can be simultaneously utilized for sequestration of specific small-molecule analytes.

2.3 Membrane Formation and Grafting

2.3.1: Additional Background on Membrane Grafting

Membranes can consist of different material types: organic, inorganic, and hybrid (both organic and inorganic) components.[1-2] Certain membrane types, such as inorganic-based ceramic membranes, utilize a lot of resources and complex techniques to prepare- thus making it impractical for our purpose.[3] Based on the commonly available materials, the organic materials provide the most benefits for our use due to their ease of preparation, cost-effectiveness- requires minimal resources and low-energy input, and efficiency for contaminant removal.[2] These membrane types align with our goals in reducing costs, time, use of resources such as machinery, energy, and pressure involved in water filtration. Additionally, organic polymers can be easily synthesized with simpler techniques, are low in costs, and are readily available.[2]

Given the compelling case of ultrafiltration membranes, we were presented with a challenge of preparing the membrane core using either hydrophobic or hydrophilic polymers (**Figure 2.1**). Hydrophilic polymers, like cellulose acetate, provide various advantages such as increased flux capacity because of the rapid water flow through the membrane due to its abundance of carboxylate esters present on the surface.[4] Contrarily, the rapid flux causes a decreased retention of organic molecules, which can pose an issue for water purification. One of the greatest advantages for hydrophobic membranes, like polyethersulfone (PES), is the high

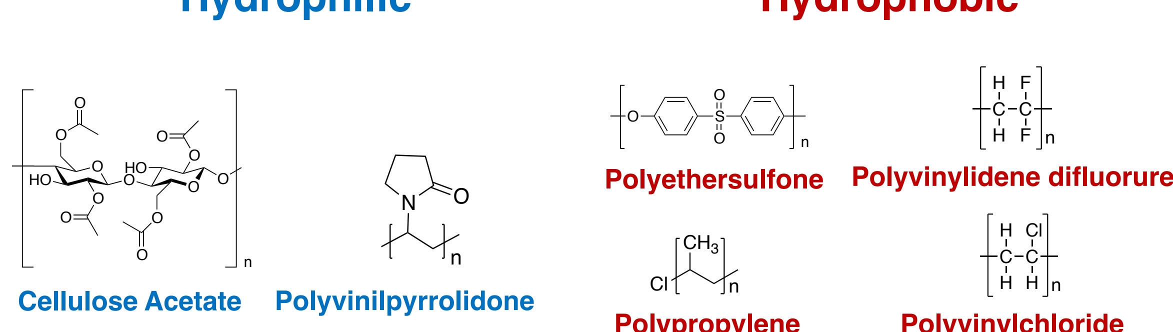


Figure 2.1: Different types of polymers used for ultrafiltration membranes. (a) Hydrophobic polymers and (b) Hydrophilic polymers.

retention of organic molecules.[5] However, its disadvantages include membrane fouling, where the increased retention capacity of organic molecules can cause the pores of the membranes to be clogged, thus hindering the passage of water.[5] The phenomena of how these different polymeric membranes operate can be easily explained because of the intermolecular forces happening between the membrane and the feed solution. Hydrophilic polymers have a tendency to mix, dissolved and/or be wetted by water.[6] Due to this, there is not much interaction with the hydrophobic organic contaminants in the water and are able to pass though the membrane easier and are not further detained by the membrane.[7] Hydrophobic polymers have the opposite effect, because of their tendency to repel water. In this case, the hydrophobic organic molecules that are present in the contaminated water are able to interact with the membrane and have greater retention.[8]

We propose that our membrane scaffold be composed of a composite design that has dual hydrophobic and hydrophilic characteristics. Advantageously, this will impart high flux and increased retention.[9-11] The only disadvantage would be that these qualities will only manifest at certain compositions. However, preparing the membranes from scratch will allow us to tune the composition for our membranes.

Because of the aforementioned scenarios, we decided to move forward with a membrane core made of PES. In addition to the high retention of organic molecules, the PES polymer possess selective permeability, mechanical stability, and chemical resistance.[10 12] PES has a high glass transition temperature between 190 and 230 ºC and is really tough.[12] For its hydrophilic counterpart, we envisioned functionalizing the PES polymer with some carboxylic acid functional groups (**Figure 2.2**). This covalent attachment would generate a membrane with dual hydrophilic and hydrophobic characteristics. There have been various reports of having copolymers made with acrylates: acrylic acid, methyl acrylate, methacrylic acid (MAA), etc.[13-14]

Methacrylic acid

Acrylic acid

Acrylates

Methyl acrylate

Figure 2.2: Common carboxylic acid-derived polymers used for copolymer formation.

We hypothesized that the addition of a covalently bound methacrylic acid to the membrane would provide favorable selectivity-permeability characteristics,[15] and more importantly, it would offer a point of attachment for the amine-modified aptamer molecules. Through looking into different surface modification techniques, we investigated the merits of surface coating, chemical cross-linking, and polymer grafting.[16-17] Polymer grafting offered the most sustainable option because this technique generates radicals by the simple addition of chemical initiators or light irradiation.[5] Cross-linking requires the use of specific chemicals called crosslinking reagents that can be very expensive and pose several hazards to the environment.[18] Polymeric coatings require expensive and difficult and complex techniques, making it not feasible for our study. In pursuit of polymer grafting, we explored the decision of generating radicals by light irradiation or chemical initiators. Light irradiation requires complicated equipment and strict operation, while chemical

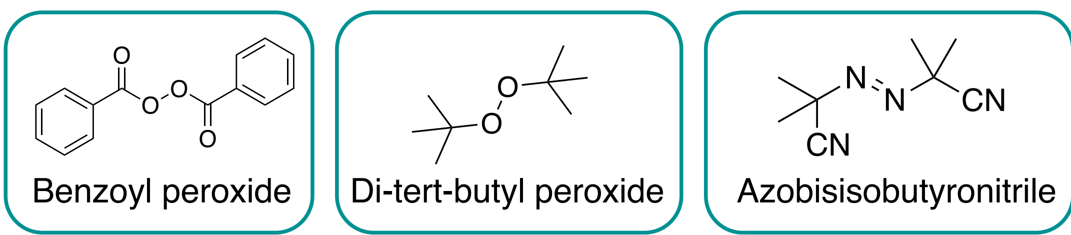


Figure 2.3: Most common radical initiators used for grafting copolymers.

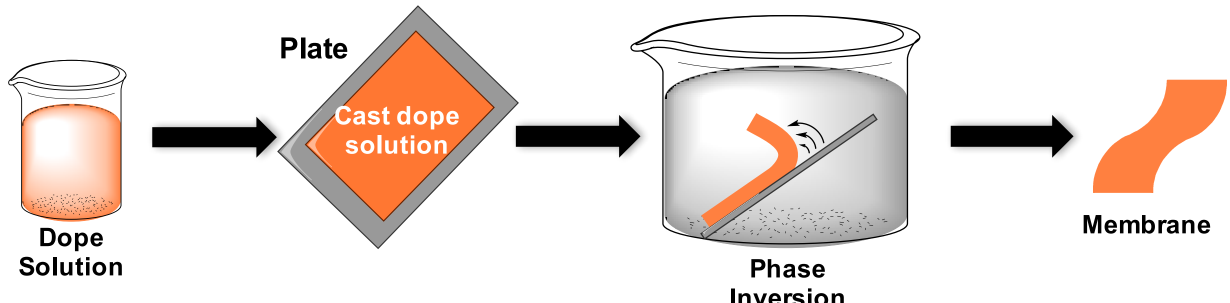
initiators can be inserted into the heterogeneous mixture of polymers to allow grafting to occur.[19] Consequently, we decided to move forward with grafting by radical initiators [20] (**Figure 2.3**).

2.3.2: *Fabrication and Standardization of Circular Membranes*

Our initial membrane design consisted of preparing the membrane with the carboxylic acids functional groups embedded on the membrane, prior to preparing the membrane. There had been recent studies of PES flakes and pellets being grafted with methacrylic acid.[21] We followed one such procedure and unfortunately, we could not reproduce these results or were able to characterize the polymer formation. We theorize that the low surface area of the pellets would not allow for radical formation, thus hindering the coupling reaction. For this reason, we decided to split the membrane fabrication process by allowing the membrane to form first, followed by grafting with carboxylic acids. For making the membrane, we were presented with different methods of membrane preparation including sintering, stretching, track-etching, template-leaching and phase inversion (**Table 2.1**).[22-23] We chose to pursue the phase inversion process due to the efficient and simple technique: precipitation of the polymer caused by the exchange of solvent and non-solvent without the need for any expensive machinery or chemicals (**Scheme 2.1**).[24]

Technique	Characteristics
Sintering	Compacting solid mass by heat or pressure
Stretching	Deformation of Polymer by cold/hot stretch cycles
Track-etching	Irradiating polymer with particles followed by chemical treatment
Template-leaching	Use of leaching component followed by acidic or enzymatic treatment
Phase Inversion	Precipitation caused by exchange of solvent and non-solvent

Table 2.1 Characteristics of different membrane formation techniques.



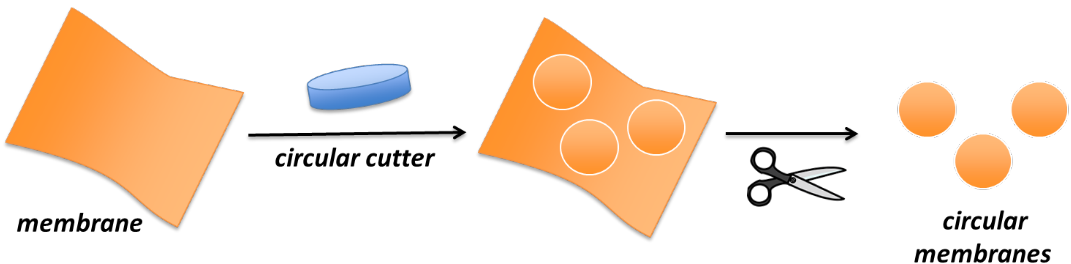
Scheme 2.1: Membrane formation by phase inversion process. The dope solution containing the hydrophobic polymer and additives, are casted on a plate. The sequential submersion in water precipitates the polymer forming the membrane.

One important property of this procedure is that we can add additives to this dope solution with the purpose of pore formation. Additives control pore formation and pore interconnectivity. [24] Different additives can be added, but one of the most common is polyethylene glycol (PEG). Depending on the size and concentration of the PEG in the homogeneous dope solution, we can achieve different purification responses. If we increase the PEG concentration in the dope solution, this increases the pore size and can allow for a greater flux. If we increase the PEG molecular weight, we can increase the membrane density allowing for solute rejection. This flexibility in membrane design can be handy depending on the water source we are decontaminating. We decided to move forward with PEG 2000 MW, as that is the molecular weight that provides the best flux due to the formation of macrovoids.[21]

We developed a standardized procedure for membrane casting, as we discovered that casting membranes by hand resulted in unsymmetrical membranes. We achieved this standardization via an Elcometer® 3580/4 casting knife film applicator and we ensured that our membranes were always stored in fresh water.

For our studies moving forward, the membranes were cut in a circular fashion due to our use of a stirred cell filtration system (**Scheme 2.2**). All of our studies were executed using an Amicon® Stirred Cell Filtration System 50 mL. This system requires a membrane diameter of 44.5

mm or 1.752 in, so we decided to use a circular cutter of 1.75 in of diameter in our sheet of membrane.



Scheme 2.2: General procedure for circular membrane formation.

For us to compare the properties of circular membranes with each another, we developed a standardization procedure. We determined that standardizing based on an output property: in terms of flux would be ideal. We determined a flux range most commonly seen in the different membranes, and only the membranes that would fall within that range would be further used. We took an initial test of n= 20 membranes and measured flux in triplicate. From the average results of each of the membranes, the mode of the averages was taken and the range was established by having ± 3 seconds (**Appendix Table 2.1**). The mode was 20.5 s for 20 mL of water filtered and the range was established at 17.5- 23.5 s (**Figure 2.4**).

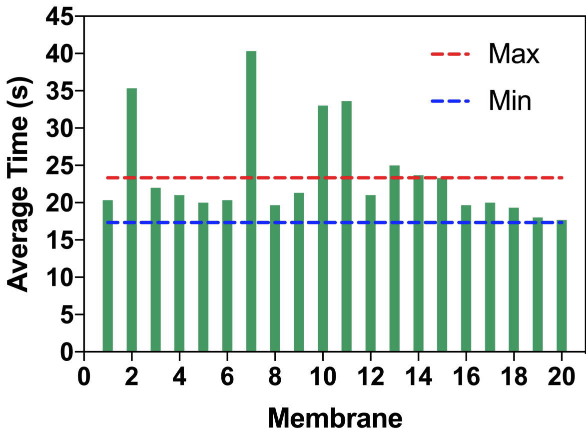
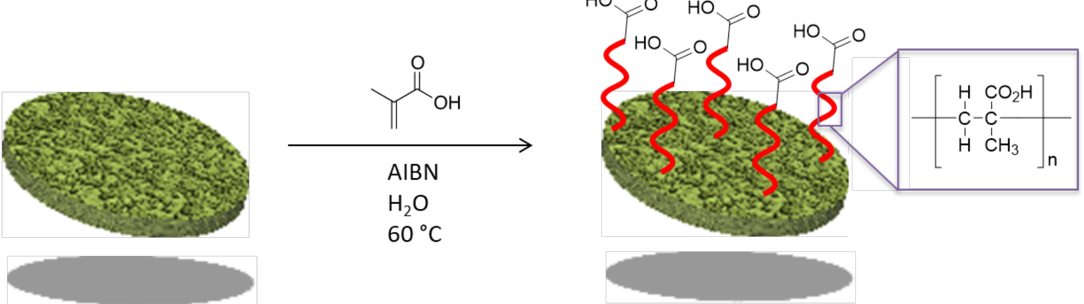


Figure Error! No text of specified style in document.**4:** Graph containing the range of the accepted time for the different membranes tested. Membranes 2, 7, 10, 11, 13 were not carried forward.

The membranes that within the accepted flux range were carried forward for grafting experiments.

2.3.3: Developing the Membrane Grafting Procedure

Grafting of the circular membranes were carried out using Azobisisobutyronitrile (AIBN) as a radical initiator, methacrylic acid as the copolymer to attach to the membrane, and water as the reaction solvent (**Scheme 2.3**).



Scheme 2.3: General procedure for grafting polymethacrylic acid (pMAA) to the membranes.

We devised a method for achieving grafting without damaging the membrane. The diameter of the membrane allowed for the reaction to take place in an equivalently-sized bottom of a 125 mL Erlenmeyer flask. The convenient narrow mouth of this flask was used to place a rubber stopper allowing us to provide an inert atmosphere (to promote the radical initiation by limiting the exposure to oxygen). We provided gentle shaking by using an orbital shaker at 500 rpm and heating at 60 °C to help further facilitate the radical reaction (**Figure 2.5**).

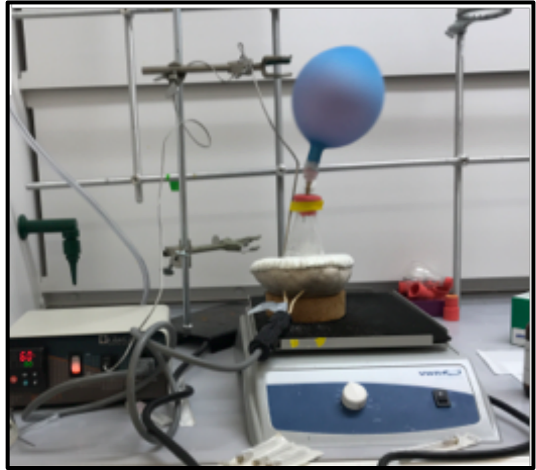


Figure 2.5: Grafting setup. The use of a heating mantle to heat the Erlenmeyer flask to 60 ºC and the use of a N_2 balloon to maintain the system in a nitrogen atmosphere. The use of the orbital shaker ensures complete mixing of components.

The subsequent propagation reaction is made possible by the addition of the methacrylic acid that generates a chain polymerization reaction. Depending on the reaction time, the pMAA chain can continue to grow because the MAA is added in excess. Once the reaction is finalized, the membrane is washed with 1 M NaOH to ensure that all unbound pMAA and other non-covalently bound species are removed from the membrane. The addition of the pMAA is possible but the exact position of covalent attachment is unknown. Proposed hypotheses for this reaction includes: (1) any free-radical fragments may abstract aromatic protons from the backbone to generate macro-radicals and (2) radicals can react with the monomer to form a growing monomeric radical, which is able to convey its radical character to the PES chain. From our procedure, the PES membrane is left to react with the AIBN for some time before the addition of MAA, so we speculate that the radicals form in the backbone of the PES. Although the exact position of the attachment is unknown, we believe that it is added to the *ortho*- position of sulfone, given that the sulfone helps stabilize the radicals (**Appendix Scheme 2.1**).

To assess the grafting process of the membrane, we performed scanning electron microscopy (SEM) on cross-sections of the membrane before and after grafting (**Figure 2.6**). This technique would give us a general idea of the pore size distribution, and confirmed the presence

Ungrafted Membrane

50 µm

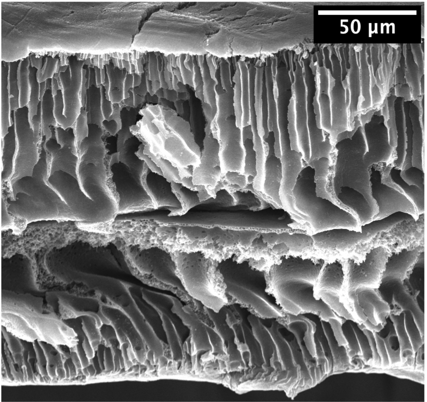


Figure 2.6: Scanning Electron Microscopy (SEM) images of ungrafted and grafted membranes showing the formation of large macrovoids and the pores of an ultrafiltration membrane. The grafting procedure did not alter the structure or the pore size distribution of the membrane.

of macrovoids due to the use of the PEG additive during membrane formation. As we can observe, the general macrovoids and structure gets retained before and after grafting, confirming that the membrane consistency remains.

We performed FT-IR with Attenuated Total Reflection (ATR) to validate the conjugation of the carboxylic acids onto our membrane. We chose this technique because our membranes were thick (i.e. micron level) and this technique is insensitive to sample thickness. Therefore, ATR provides a good measure and operates by measuring the changes that occur in an internally reflected IR beam when the beam comes in contact with the sample. The addition of the carboxylic acids to our membrane sample was observed by the presence of two distinct bands with an O-H stretch from 3300-2500 cm^{-1} and a C=O stretch from 1760-1690 cm^{-1} (**Figure 2.7**).

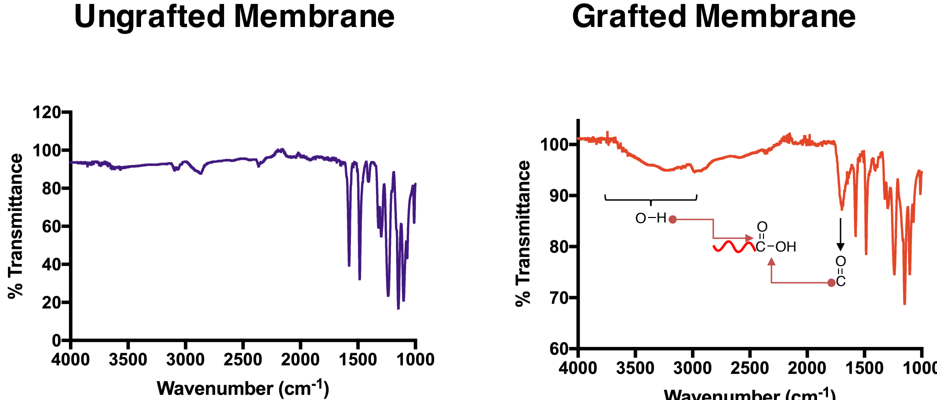


Figure 2.7: IR spectrum of grafted and ungrafted membrane showing the addition of the carboxylic acids on the membrane.

To supplement our validation for the addition of the carboxylic acid moieties to the membrane, we performed X-ray photoelectron spectroscopy (XPS) to measure the elemental composition and the differentiated chemical states of the polymer on the membrane. Elemental composition data illustrates a higher abundance of oxygen atoms in the grafted membrane compared to the ungrafted sample, supporting the presence of pMAA (**Table 2.2**). Furthermore, we measured the relative atomic percentage of the carbon components to observe different carbon electronic states (**Figure 2.8**). The graphs showing the different counts per second (CPS) of the carbon components as a function of binding energy. We observed the presence of a new

peak at 288.41 eV denoting the presence of the CO_2H group and confirming the grafting of the membrane.

Table 2.2: Table detailing the elemental composition as relative atomic percent (At%). Oxygen composition is higher on the grafted membrane, suggesting the presence of the pMAA chain.

Surface Elemental Composition (Relative Atomic Percent)			
Samples	**O**	**C**	**S**
PES Ungrafted	18.1	75.3	3.73
PES Grafted	22.2	72.1	1.1

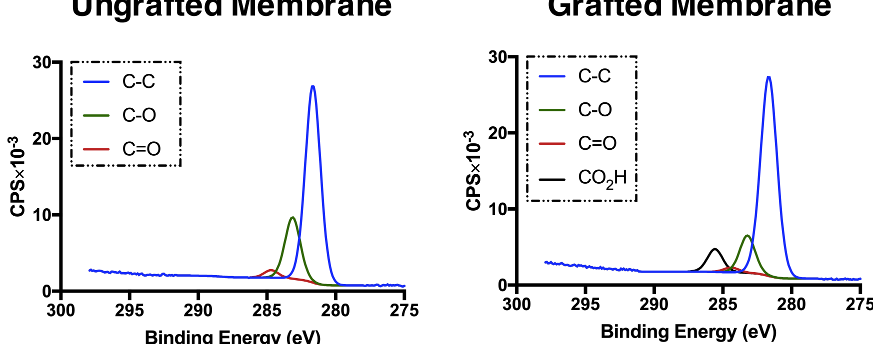


Figure 2.8: Overlaid high resolution XPS scans of the ungrafted and grafted membranes. C-C peak binding energy is corrected to 284.5 eV. The presence of the peak at 288.41 eV in the grafted membrane confirms the presence of pMAA.

We also focused on optimizing the grafting time so that would not detract from the overall flux capacity (**Appendix Table 2.2**). We discovered that a one-hour reaction time was optimal because there was only a 14 second increase from the grafted and ungrafted membrane, which has no significant effect on the overall flux (**Figure 2.9**).

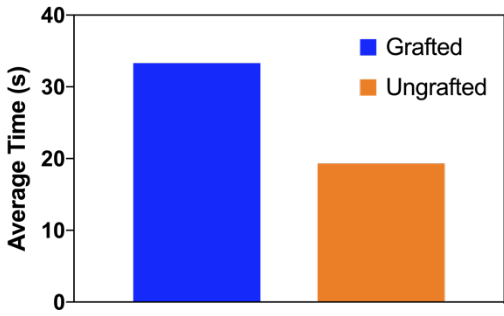


Figure 2.9: The effect of grafting on membrane flux capacity.

The successful addition of this pMAA to the membrane provides a great balance between the hydrophobicity and hydrophilicity of the membrane (**Figure 2.10**). These characteristics in conjunction with the additives used to fabricate the membrane conveys a scaffold that can potentially have increased efficiency in terms of sequestering contaminants from water once we are able to functionalize the membrane with DNA aptamers.

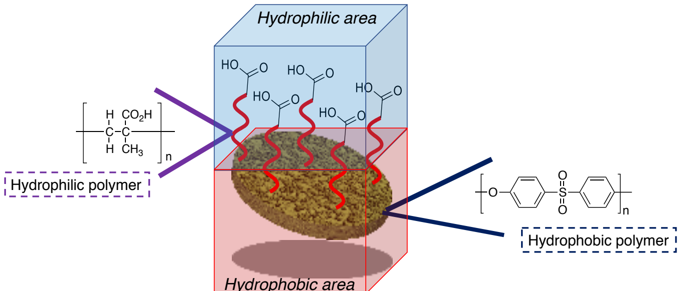


Figure 2.10: General scaffold of ultrafiltration membranes with the hydrophobic and hydrophilic portions outlined.

2.4 Membrane Functionalization with DNA Aptamers

2.4.1: *Attachment of Aminofluorescein*

In order to ensure that the DNA attachment process did not detract the reactivity or the functionality of the carboxylic acid groups, we used a surrogate molecule: aminofluorescein that contains a free amine group. The free amino group would react with the carboxylate group and form an amide bond to enable attachment. The aminofluorescein Isomer I is a great molecule for amide bond formation because it has a free amine that can allow for the reaction to occur and its

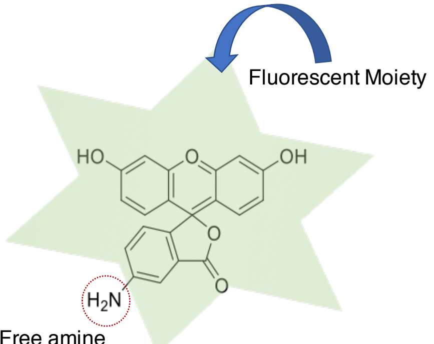
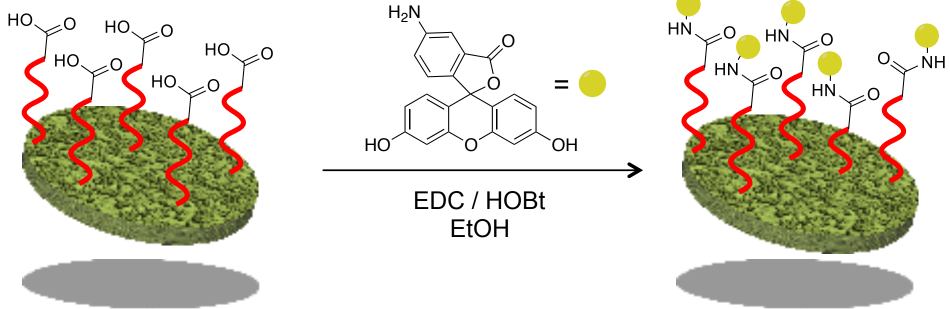


Figure 2.11: Structure of the Aminofluorescein Isomer I aptamer surrogate.

fluorescent capabilities can allow us to quantify the loading capacity of our membrane (**Figure 2.11**).

The coupling reaction was performed in ethanol with the 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and Hydroxybenzotriazole (HOBt) as coupling reagents (**Scheme 2.4**). We used ethanol because we observed that this solvent was able to dissolve aminofluorescein, but not the membrane. This reaction allowed us to observe the attachment of aminofluorescein onto the membrane by quantifying the fluorescence of the membrane after multiple wash cycles.



Scheme 2.4: Amide bond formation between aminofluorescein and the carboxylic acid groups of the membrane using EDC and HOBt as coupling reagents.

To quantify the aminofluorescein-functionalized membranes, we divided (cut) them into equal parts (**Appendix Scheme 2.2**) and weighed 5 mg of each portion and dissolved them DMSO. The aminofluorescein functionalization was determined by preparing a calibration curve and determining the amount of aminofluorescein per membrane. This experiment was accompanied by a control experiment with the aminofluorescein reaction of the ungrafted membrane to make sure that the fluorescence readout was due to covalent attachment of the aminofluorescein (**Appendix Table 2.3**). We observed fluorescence in the grafted membranes, with a yield of 12.9 $\pm$ 0.9 μmol per milligram of membrane (**Figure 2.12**).

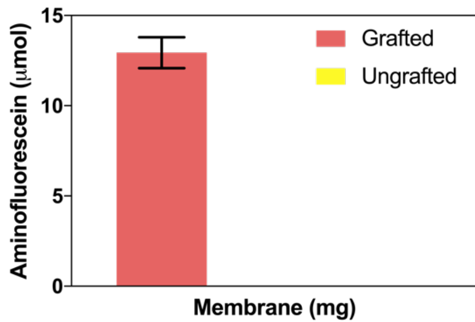
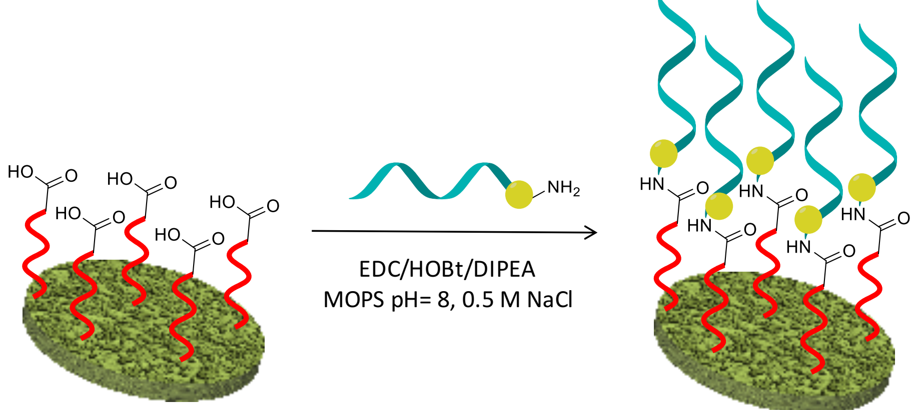


Figure 2.12: Fluorescence of the grafted and ungrafted membranes after functionalization. Error bars represent SD (n=3).

2.4.2: Attachment of DNA Aptamers

After we received confirmation that the amine-modified chemistry was possible, we examined the ability for the membrane to couple ssDNA aptamers (**Table 2.3**) to determine that the membranes were able to not discriminate in its aptamer attachment. Following a similar procedure for aminofluorescein attachment, with the exception of a MOPS buffer in lieu of ethanol to avoid any DNA denaturing (**Scheme 2.5**). We performed the same control experiments by doing the reaction with the ungrafted membrane to also ensure that the DNA coupling is due to covalent attachment and not to any non-specific binding.



Scheme 2.5: General procedure for DNA functionalization.

Table 2.3: DNA functionalization reaction with different types of aminofluorescein-labeled DNA.

Entry	NH_2	DNA loading (nmol) per mg of membrane	
		Grafted	Ungrafted
1	5'-NH_2/FAM/spacer 6-CACATCAATC-spacer 6-3'	9.92	0.327
2	5'-NH_2/spacer 6/FAM/spacer 6-CACATCAATC-spacer 6-3'	21.4	0.412
3	5'-NH_2/spacer 6/FAM/spacer 6-CACATCAATC-spacer 9-3'	10.2	0.33

Furthermore, we were able to observe that covalent attachment of the aptamer to the membrane produced the amide bond as we observed DNA loading with the grafted membrane only (**Figure 2.13**).

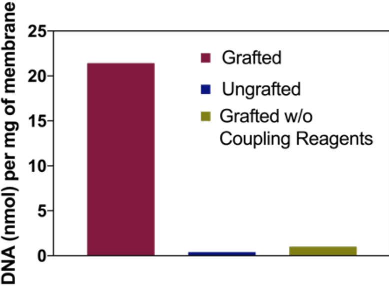


Figure Error! No text of specified style in document.**13:** DNA functionalization with three different membrane types to observe the covalent attachment of DNA.

While our DNA quantification approach detailed above is highly accurate, this characterization approach is destructive to the membranes. We focused on developing a method of quantifying ssDNA attachment without destroying the membrane. We hypothesized that we could do this by monitoring the quantity of DNA in solution as an indicator of reaction efficiency. We would take small aliquots before and after reaction and the difference in fluorescence would be a strong indicator of the amount of aptamer attached to the membrane. Since the circular membrane is of 1.75 inches in diameter, we had to find a way that the membrane can be submerged and shaken without undergoing any damage. We were able to achieve this by

inserting the membrane into a 50 mL conical tube and using an orbital shaker to ensure that the DNA solution interacted with the membrane (**Figure 2.14**).

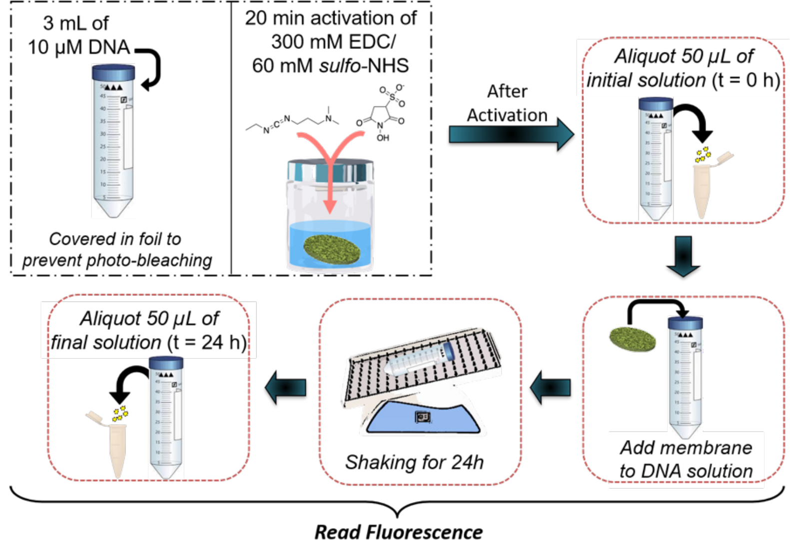


Figure Error! No text of specified style in document.**14:** General procedure for functionalizing circular membranes and monitoring fluorescence of the reaction.

We also observed that conjugation using *sulfo*-NHS and EDC under the conditions described by Li et al.[25] produced the best results over other conditions, such as EDC/HOBt/ DIPEA and DMT-MM, which resulted in no detectable reaction. We also wanted to see if different structural aptamers affected the yields of the conjugation of DNA onto the aptamer and investigated this using two different kinds of DNA: a primary structure based polyT aptamer and another aptamer known to form a secondary structure. This is central to our fundamental studies because we expect yields to vary based on their conformational structure and the amount of space aptamers could potentially occupy on the membrane. For contaminant sequestration purposes, we would hope to achieve reasonable yields for aptamers that adapt a secondary structure. The addition of the aptamers to the membrane was achieved with fair yields, with a lower amount of yields for aptamers that adopt a secondary structure (**Figure 2.15**).

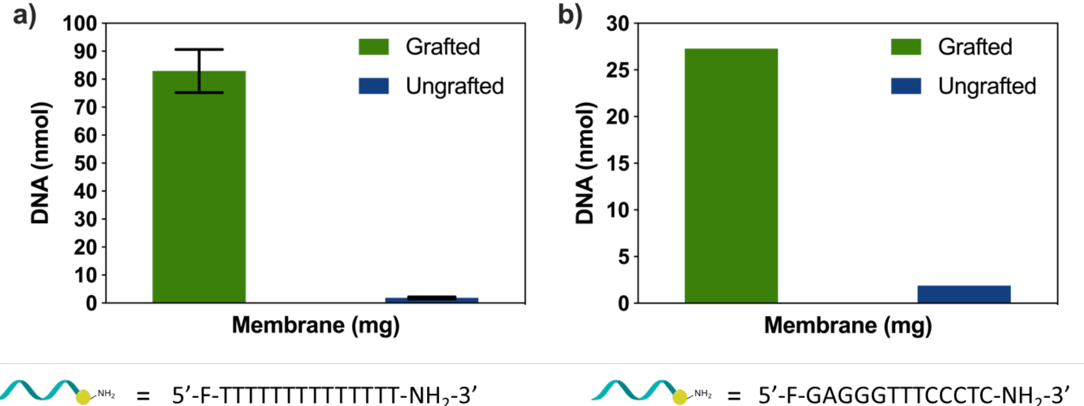


Figure 2.16: Functionalization of structurally different ssDNA including a) polyT aptamer and b) a random sequence known to form a secondary structure using the optimized coupling method. Error bars on a) represent SD (n=3).

The addition of the BPA aptamer (adapted from the truncation that Son and coworkers)[26] was coupled with an amine: NH_2 group in the 3' end, and a FAM fluorophore on the 5' end to enable coupling with the carboxylic acid and quantify aptamer loading. We observed that 43% of the anti-BPA aptamer was conjugated to the membrane (**Figure 2.16**).

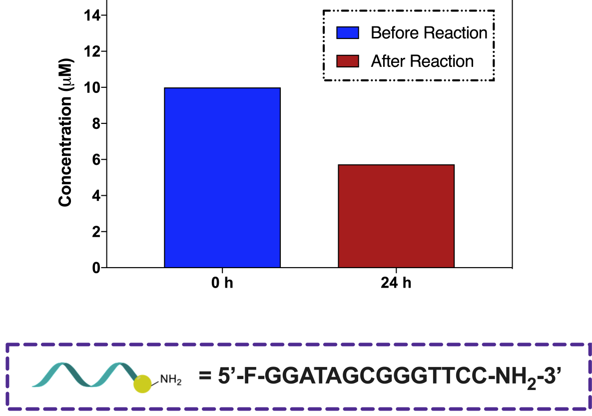


Figure 15: The decrease in DNA concentration in the supernatant was used as a metric to quantify the amount of BPA aptamer attached to the membrane. The sequence of the BPA aptamer used for reaction consist of a 14-base ssDNA with amino and FAM modifications.

We were also curious to observe the effect that functionalization of this BPA DNA aptamer would have on the membrane's overall flux. We ran flux on the membrane before and after functionalization and observed that the addition of DNA poses no effect on flux capacity (**Figure 2.17**).

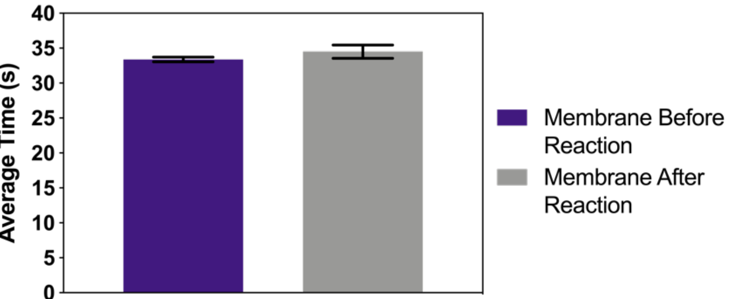


Figure 2.17: Effect of BPA aptamer functionalization on membrane flux. Error bars represent SEM (n=3).

With these results, we were able to develop a procedure for functionalizing the grafted membrane with DNA aptamers. The process for developing the aptamer-functionalized membrane consist of three major components: the hydrophobic PES polymer-based aptamer core, the hydrophilic pMAA chain, and the aptamer which generates an amide bond with the carboxylic acids (**Figure 2.18**).

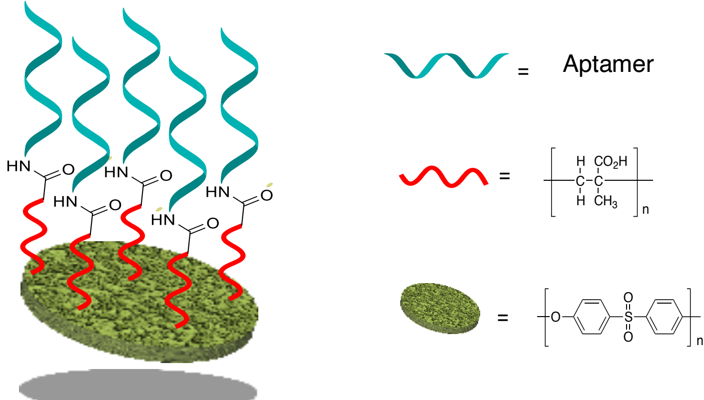


Figure 2.18: The components that the aptamer-functionalized membrane incorporates. It consists of three major components: aptamer, pMAA and PES.

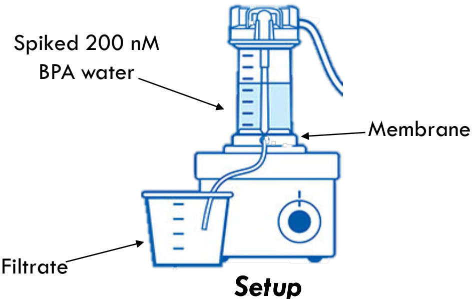
2.5 Depletion of BPA from Water

2.5.1: Depletion of BPA by Membrane

Once the aptamer functionalized membranes were prepared, we needed to observe its application into removing the small-molecule of interest, which in this case is BPA. To achieve this, we first tested BPA removal efficiency by preparing a feed solution of 200 nM of BPA, the maximum concentration that is routinely found in water sources. We were able to observe how

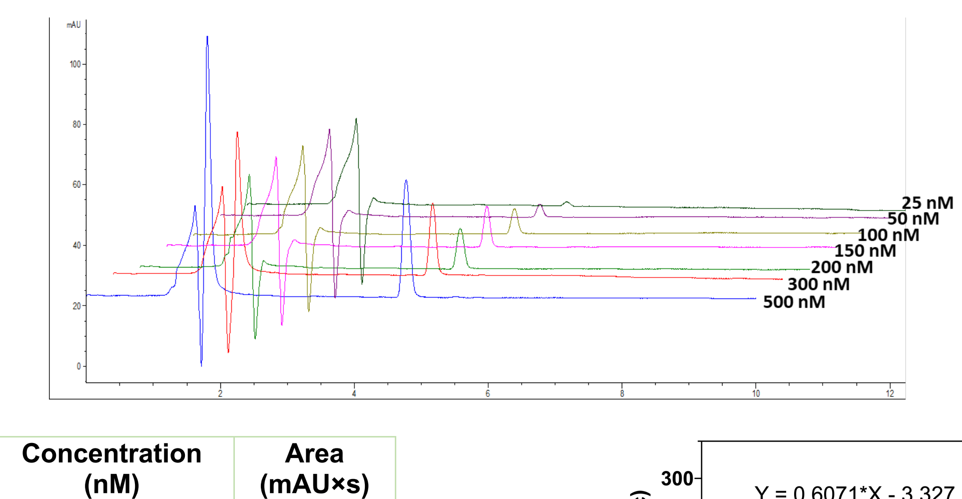
much BPA was depleted by filtering BPA-contaminated water though the membrane and using a stirred cell filtration system (**Scheme 2.6**).

We analyzed BPA removal efficiency by quantifying the permeate concentration using



Scheme 2.6: General setup for BPA filtration experiments.

HPLC. We were able to measure the area under the curve for each of the different water types containing BPA and we formulated a calibration curve that helped us quantify the amount of BPA that the membrane depleted (**Figure 2.19**)



Concentration (nM)	Area (mAU×s)
500	309.56
300	167.361
200	107.049
150	91.8348
100	58.1596
50	29.8996
25	17.2473

Figure 2.19: HPLC method for BPA detection, including calibration curve.

We initiated our investigation by examining that the sequestration of BPA was only due to the presence of the aptamers and not by non-specific adsorption of the membrane. We validated this by performing filtration experiments with the grafted, non-functionalized membrane. We filtered the BPA-spiked water through the grafted membrane and observed no depletion of BPA (**Figure 2.20**) which suggested that any potential depletion could be due to the aptamers.

Following these results, we observed the ability of the aptamer-functionalized membrane

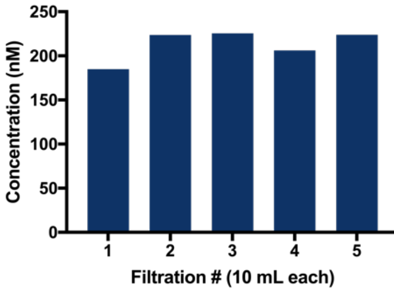


Figure 2.20: BPA depletion of the grafted membrane. There is no change in the concentration, demonstrating that BPA cannot be depleted by the grafted membrane itself.

to remove BPA in standard Milli-Q water spiked with 200 nM BPA. The water was filtered through the aptamer-functionalized membrane (**Figure 2.21**) and we were able to observe depletion of the BPA to a concentration below the guidelines that the Environmental Protection Agency has adopted for safe drinking water.

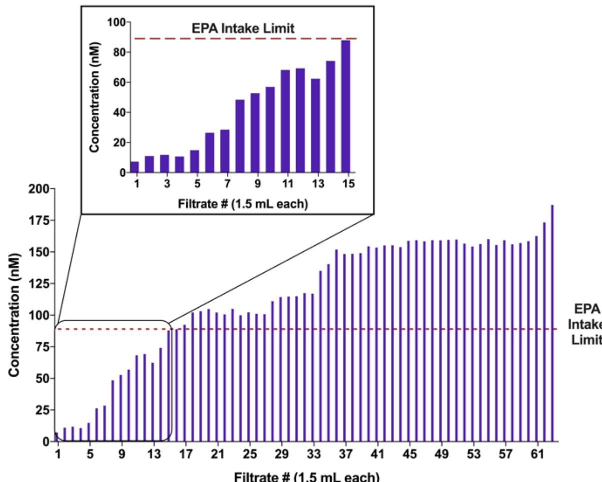


Figure 2.21: Breakthrough curve depicting BPA depletion of Milli-Q water as a function of the volume of water filtered, using a 200 nM BPA feed solution. Parameters: 1 bar of pressure 1.5 mL of filtrate collection volumes, Amicon Stirred Cell 50 mL apparatus.

We were able to observe that a membrane of 4.5 cm in diameter and 108 mg in weight with an estimated 12.5 nmol of functionalized aptamer was able to deplete 6.4 nmol of BPA prior to sorbent exhaustion. Of this total depletion, 3.7 nmol of BPA was removed from the 27 mL of water filtered before reaching the EPA intake limit, and an additional 2.7 nmol of BPA was removed before reaching sorbent exhaustion. While this is still below quantitative binding, there are several reasonable explanations for this: (1) although the affinity of this aptamer is quite high (Kd = 8 nM), this still does not enable 100% occupancy of binding sites at equilibrium; (2) a portion of immobilized aptamers may not be in the optimal conformation for BPA binding; (3) steric hindrance and/or cross-hybridization between adjacent aptamers on the surface may disrupt the function of some aptamers.

To further demonstrate practical utility, we explored the ability of our membranes to function with natural water samples by obtaining lake water from a local source: Chandler Lake-Lullwater Preserve at Emory University. No BPA was detected in this initial lake water sample, so we spiked it to a known concentration (200 nM) in the same manner as the Milli-Q water experiments. The feed solution was subjected to identical conditions as our previous experiments, and we did not further pretreat the sample during our analysis. We filtered this

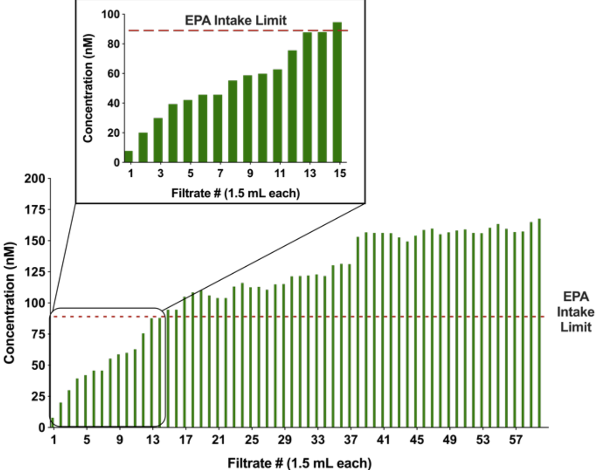


Figure 2.22: Breakthrough curve showing BPA depletion of lake water as a function of the volume of water filtered, using a 200 nM BPA feed solution. Parameters: 1 bar of pressure, 1.5 mL of filtrate collection volumes, Amicon Stirred Cell 50 mL apparatus.

spiked-lake water and achieved similar performance to that observed for Milli-Q water, with the

aptamer-functionalized membrane being able to deplete 6.1 nmol of BPA prior to sorbent exhaustion (**Figure 2.22**).

2.5.2: *Aptamer Specificity to BPA and Other Removal of Contaminants*

We also wanted to observe the aptamer specificity to BPA, as the removal of other small-molecule contaminants would hinder the capabilities of the aptamers to remove BPA. To test this, we obtained other common organic contaminants of water: diethylstilbestrol, a BPA analogue and 4-Chlorophenol, a common phenolic contaminant.[26] We spiked the water in the same way that we did for the BPA experiments and we filtered some aptamer-functionalized membranes through these solutions. We observed that the concentrations of these contaminants remain unaltered after filtering (**Figure 2.23**). These results suggest that attachment of the aptamer to the membrane does not interfere with aptamer selectivity and that the aptamer-functionalized membranes are able to specifically sequester the BPA target.

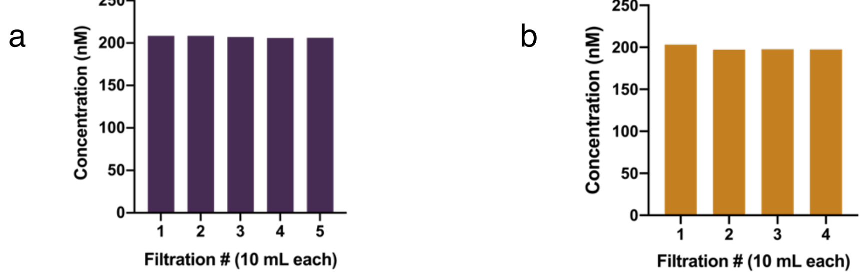
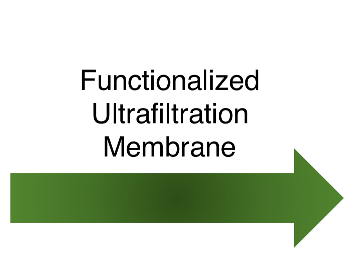


Figure 2.23: Depletion of other small-molecule contaminants by aptamer-functionalized membranes. (a) diethylstilbestrol and (b) 4-chlorophenol.

We performed a qualitative analysis on the unfiltered and filtered lake water to investigate the ability of the functionalized membrane to remove larger contaminants. The filtration process was able to remove particulates and other large matter from the sample, as noted by the visible change in the water (**Figure 2.24**).

a)



b)

			Concentration ppm (parts per million)												
Sample	**Hardness**	**pH**	**Al**	**B**	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**	**Na**	**P**	**Si**	**Zn**
Lake Water	43.99	6.7	<0.10	0.031	12.27	<0.05	<0.10	4.31	3.24	<0.05	<0.01	6.25	<0.02	3.00	<0.05
Filtered Water	43.10	7.0	<0.10	0.031	11.93	<0.05	<0.10	4.14	3.23	<0.05	<0.01	6.88	0.024	3.34	<0.05

c)

	ppm (parts per million)							ppb (parts per billion)		µS/cm
Sample	**Alkalinity**	**CO_2**	**Cl**	**F**	**NO_3^-**	**PO_4**	**SO_4**	**Cr**	**Ni**	**Conductivity**
Lake Water	54.00	20.11	7.08	<0.17	<0.19	<1.00	1.43	<10	<10	127.9
Filtered Water	52.00	9.49	7.12	<0.17	<0.19	<1.00	1.46	<10	<10	127.8

Figure 2.24: a) Qualitative difference in the lake water sample before and after filtration with the functionalized ultrafiltration membrane. In addition to removing BPA we can observe the removal of large particulates and other contaminants. b) Expanded water test performed by the Agricultural and Environmental Services Laboratories at the University of Georgia. Colors denote the different types of tests performed: Peach: Basic Water Test, Pink: Anions, Blue: Soluble Salts, Gold: Alkalinity, Green: Heavy Metals.

With the successful removal of BPA from water, we have developed a technology that has the ability of removing small molecules from water. This platform has the ability of removing any toxin of interest, given appropriate aptamer attachment.

2.6 Regeneration of the Aptamer-Functionalized Membrane

Another interesting application of the aptamer-functionalized membranes is their ability to regenerate and restore their structure and function after filtration. This would enable multiple uses and could potentially pave the way to allow recovery of valuable small-molecule analytes in the future. The retentate of the same membrane from the BPA depletion experiment described above was washed away with water at 65 °C because this temperature disrupts the aptamer structure without damaging the membrane. After regeneration, the membrane depletion capacity was reevaluated using 200 nM BPA-spiked water as the feed solution. We observed a slight decrease in BPA removal capacity after the first regeneration, but the capacity then remained stable over multiple further regeneration cycles, demonstrating the reusability of the membranes (**Figure 2.25**).

1. **First regeneration:**

   Volume of water filtered before reaching EPA limit: 10.5 mL (17.9 s) (Filtrate #: 1-7)

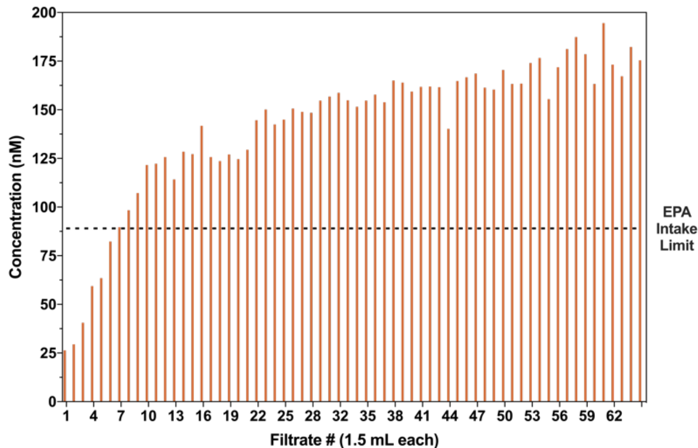
   nmol of BPA removed: 1.52

   Volume to breakthrough: 21 mL (36 s)

   Volume of water filtered before reaching sorbent exhaustion: 42 mL (63 s) (Filtrate #: 1-28)

   nmol of BPA removed: 2.18

   Total BPA removed: 3.7 nmol



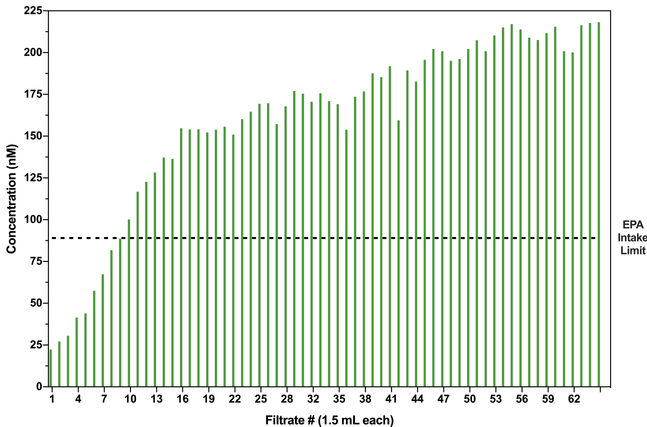
2. **Second regeneration**

Volume of water filtered before reaching EPA limit: 13.5 mL (23 s) (Filtrate #: 1-9)
nmol of BPA removed: 2.01

Volume to breakthrough: 34.5 mL (59 s)

Volume of water filtered before reaching sorbent exhaustion: 69 mL (103.5 s) (Filtrate #: 1-46)
nmol of BPA removed: 2.05

Total BPA removed: 4.06 nmol



3. **Third regeneration**

Volume of water filtered before reaching EPA limit: 15 mL (22.5 s) (Filtrate #: 1-10)

nmol of BPA removed: 2

Volume to breakthrough: 28.5 mL (48.5 s)

Volume of water filtered before reaching sorbent exhaustion: 55.5 mL (83.5 s) (Filtrate #: 1-37)
nmol of BPA removed: 2.47
Total BPA removed: 4.47 nmol

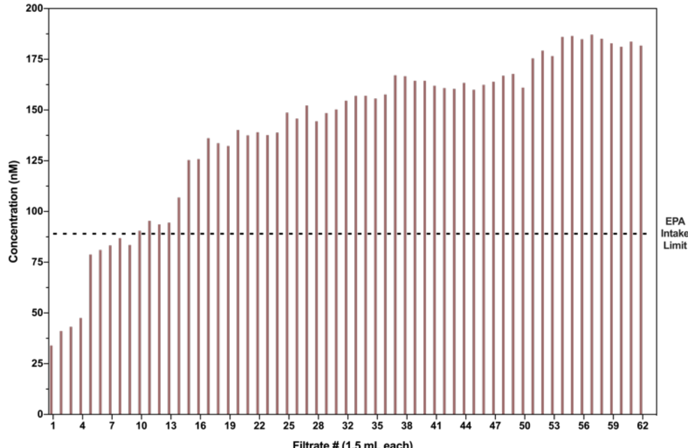


Figure 2.25: Regeneration of the aptamer functionalized membrane after multiple regeneration cycles. There is a slight decrease in BPA removal capacity after the first regeneration, but the capacity then remained stable through the rest regeneration cycles.

2.7 Conclusions

The ability to sequester specific small molecules of interest would enable the removal of dangerous contaminants from the environment and make possible the recovery of precious compounds and other metals found in contaminated water sources. Aptamers offer a promising solution to this challenge because they are able to bind to a specific small molecule or a set of related molecules with high affinity and they can be reversibly denatured, allowing for analyte recovery when preferred. Ultrafiltration membranes serve as a convenient scaffold for the aptamers as they are easy to produce, have high water permeability, and can also be used to remove macroscale contaminants. Here, we demonstrate the synthesis, characterization, and use of aptamer-functionalized ultrafiltration membranes for the removal of small molecules from water. As an initial demonstration, we show that BPA can be depleted and that the membranes can be recycled by reversible denaturation of the aptamers. We demonstrate practical utility by achieving depletion of BPA from a natural lake water sample. While our initial proof-of-concept example is focused on BPA removal, aptamers can be generated for a wide variety of small-molecule (and protein) analytes.

2.8 Appendix

Supplement- Chapter 2: Sequestration of Bisphenol A by the Aptamer-Functionalized Membrane

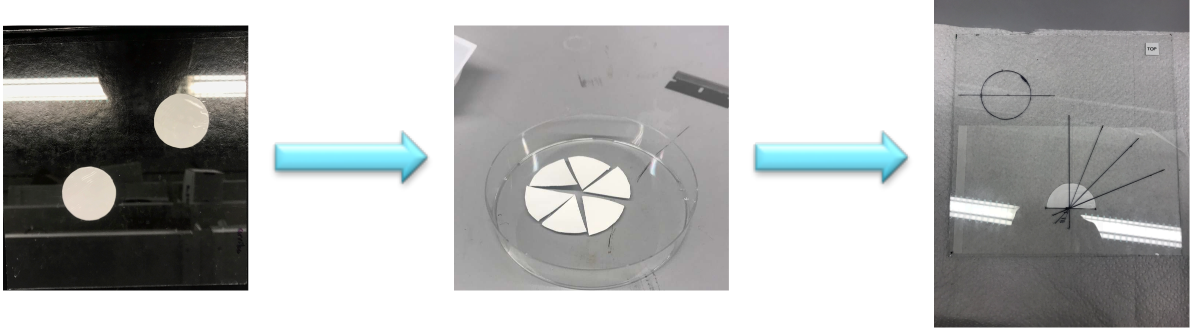
Table 2.1 Flux experiments for standardization of membranes. Time of filtration for the different membrane set tested for 20 mL.

Membrane	Volume read (mL)	Δv (mL)	t1 (s)	t2 (s)	t3 (s)	Average time (s)
1	40-20	20	18	20	23	20.33
2	40-20	20	33	35	38	35.33
3	40-20	20	19	23	24	22.00
4	40-20	20	19	21	23	21.00
5	40-20	20	18	20	22	20.00
6	40-20	20	19	20	22	20.33
7	40-20	20	34	41	46	40.33
8	40-20	20	17	20	22	19.67
9	40-20	20	16	19	29	21.33
10	40-20	20	33	33	33	33.00
11	40-20	20	32	31	38	33.60
12	40-20	20	19	21	23	21.00
13	40-20	20	24	25	26	25.00
14	40-20	20	21	24	26	23.67
15	40-20	20	23	23	24	23.30
16	40-20	20	18	20	21	19.67
17	40-20	20	18	20	22	20.00
18	40-20	20	19	19	20	19.33
19	40-20	20	19	17	18	18.00
20	40-20	20	17	17	19	17.67

Scheme 2.1. Proposed grafting mechanism for the addition of pMAA chains to the PES membrane. (a) radical initiator formation and (b) pMAA grafting onto PES.

Table 2.2 Different flux times for the grafted and ungrafted membranes as they filter 20 mL of water.

Membrane	Volume read (mL)	Δv (mL)	t1 (s)	t2 (s)	t3 (s)	Average time (s)
Grafted	40-20	20	33	33	34	33.33
Ungrafted	40-20	20	19	19	20	19.33



Scheme 2.2 Overview of the membrane cutting procedure prior to functionalizing the membranes with aminofluorescein.

Table 2.3 Loading capacity of aminofluorescein for different batches of ultrafiltration membranes. Membranes 1-3 are grafted and membranes 4-6 are ungrafted. We only observe fluorescence in the grafted membranes.

	Fluorescence	Concentration (mM)	mmol	mmol per mg	µmol per mg
Membrane 1	1635.33	40.89	0.0047	0.0123	12.28
Membrane 2	1676.00	42.10	0.0048	0.0126	12.64
Membrane 3	1819.00	46.32	0.0053	0.0139	13.91
Membrane 4	248.00	0	-	-	-
Membrane 5	240.33	0	-	-	-
Membrane 6	239.00	0	-	-	-

2.9 References

1. Geise, G. M.; Lee, H. S.; Miller, D. J.; Freeman, B. D.; McGrath, J. E.; Paul, D. R., Water purification by membranes: The role of polymer science. *Journal of Polymer Science Part B: Polymer Physics* **2010,** *48* (15), 1685-1718.

2. Lee, A.; Elam, J. W.; Darling, S. B., Membrane materials for water purification: design, development, and application. *Environmental Science: Water Research & Technology* **2016,** *2* (1), 17-42.

3. Fane, A. G.; Wang, R.; Hu, M. X., Synthetic Membranes for Water Purification: Status and Future. *Angewandte Chemie International Edition* **2015,** *54* (11), 3368-3386.

4. Singh, R., Chapter 2 - Water and Membrane Treatment. In *Membrane Technology and Engineering for Water Purification (Second Edition)*, Singh, R., Ed. Butterworth-Heinemann: Oxford, 2015; pp 81-178.

5. Alenazi, N. A.; Hussein, M. A.; Alamry, K. A.; Asiri, A. M., Modified polyether-sulfone membrane: a mini review. *Des Monomers Polym* **2017,** *20* (1), 532-546.

6. Hatakeyama, H.; Hatakeyama, T., Interaction between water and hydrophilic polymers. *Thermochimica Acta* **1998,** *308* (1), 3-22.

7. Thakur, V. K.; Voicu, S. I., Recent advances in cellulose and chitosan based membranes for water purification: A concise review. *Carbohydrate Polymers* **2016,** *146*, 148-165.

8. Werber, J. R.; Osuji, C. O.; Elimelech, M., Materials for next-generation desalination and water purification membranes. *Nature Reviews Materials* **2016,** *1* (5), 16018.

9. Tian, E. L.; Zhou, H.; Ren, Y. W.; mirza, Z. a.; Wang, X. Z.; Xiong, S. W., Novel design of hydrophobic/hydrophilic interpenetrating network composite nanofibers for the support layer of forward osmosis membrane. *Desalination* **2014,** *347*, 207-214.

10. Wavhal, D. S.; Fisher, E. R., Hydrophilic modification of polyethersulfone membranes by low temperature plasma-induced graft polymerization. *Journal of Membrane Science* **2002,** *209* (1), 255-269.

11. Wang, Y.; Kim, J.-H.; Choo, K.-H.; Lee, Y.-S.; Lee, C.-H., Hydrophilic modification of polypropylene microfiltration membranes by ozone-induced graft polymerization. *Journal of Membrane Science* **2000,** *169* (2), 269-276.

12. Rahimpour, A.; Madaeni, S. S., Polyethersulfone (PES)/cellulose acetate phthalate (CAP) blend ultrafiltration membranes: Preparation, morphology, performance and antifouling properties. *Journal of Membrane Science* **2007,** *305* (1), 299-312.

13. Tu, M.-M.; Xu, J.-J.; Qiu, Y.-R., Surface hemocompatible modification of polysulfone membrane via covalently grafting acrylic acid and sulfonated hydroxypropyl chitosan. *RSC Advances* **2019,** *9* (11), 6254-6266.

14. Ng, L. Y.; Ahmad, A.; Mohammad, A. W., Alteration of polyethersulphone membranes through UV-induced modification using various materials: A brief review. *Arabian Journal of Chemistry* **2017,** *10*, S1821-S1834.

15. Deng, B.; Yang, X.; Xie, L.; Li, J.; Hou, Z.; Yao, S.; Liang, G.; Sheng, K.; Huang, Q., Microfiltration membranes with pH dependent property prepared from poly(methacrylic acid) grafted polyethersulfone powder. *Journal of Membrane Science* **2009,** *330* (1), 363-368.

16. Nady, N.; Franssen, M. C. R.; Zuilhof, H.; Eldin, M. S. M.; Boom, R.; Schroën, K., Modification methods for poly(arylsulfone) membranes: A mini-review focusing on surface modification. *Desalination* **2011,** *275* (1), 1-9.

17. Kang, M. S.; Chun, B.; Kim, S. S., Surface modification of polypropylene membrane by low-temperature plasma treatment. *Journal of Applied Polymer Science* **2001,** *81* (6), 1555-1566.

18. Minto, F.; Gleria, M.; Pegoretti, A.; Fambri, L., Blending, Grafting, and Cross-Linking Processes between Poly(ethylene oxide) and a (4-Benzoylphenoxy)~0.5(Methoxyethoxyethoxy)~0.5Phospha- zene Copolymer. *Macromolecules* **2000,** *33* (4), 1173-1180.

19. Rahimpour, A.; Madaeni, S. S.; Zereshki, S.; Mansourpanah, Y., Preparation and characterization of modified nano-porous PVDF membrane with high antifouling property using UV photo-grafting. *Applied Surface Science* **2009,** *255* (16), 7455-7461.

20. Matabola, K. P.; Bambo, M. F.; Sikhwivhilu, K.; Vatsha, B.; Moutloali, R. M., Chemical Grafting of Polystyrene Sodium Sulfonate (PSS) onto Polyethersulfone (PES) Powder and Effect on the Characteristics of the Resultant Ultrafiltration Membranes. *Materials Today: Proceedings* **2015,** *2* (7), 3957-3963.

21. Shi, Q.; Su, Y.; Ning, X.; Chen, W.; Peng, J.; Jiang, Z., Graft polymerization of methacrylic acid onto polyethersulfone for potential pH-responsive membrane materials. *Journal of Membrane Science* **2010,** *347* (1), 62-68.

22. Song, Y.; Sun, P.; Henry, L. L.; Sun, B., Mechanisms of structure and performance controlled thin film composite membrane formation via interfacial polymerization process. *Journal of Membrane Science* **2005,** *251* (1), 67-79.

23. Reverchon, E.; Cardea, S., Formation of cellulose acetate membranes using a supercritical fluid assisted process. *Journal of Membrane Science* **2004,** *240* (1), 187-195.

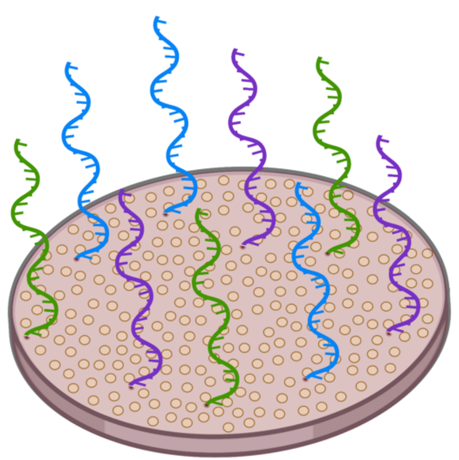
24. Boom, R. M.; Wienk, I. M.; van den Boomgaard, T.; Smolders, C. A., Microstructures in phase inversion membranes. Part 2. The role of a polymeric additive. *Journal of Membrane Science* **1992,** *73* (2), 277-292.

25. Li, Y.; Gabriele, E.; Samain, F.; Favalli, N.; Sladojevich, F.; Scheuermann, J.; Neri, D., Optimized Reaction Conditions for Amide Bond Formation in DNA-Encoded Combinatorial Libraries. *ACS Combinatorial Science* **2016,** *18* (8), 438-443.

26. Lee, E.-H.; Lim, H. J.; Lee, S.-D.; Son, A., Highly Sensitive Detection of Bisphenol A by NanoAptamer Assay with Truncated Aptamer. *ACS Applied Materials & Interfaces* **2017,** *9* (17), 14889-14898.

Chapter 3

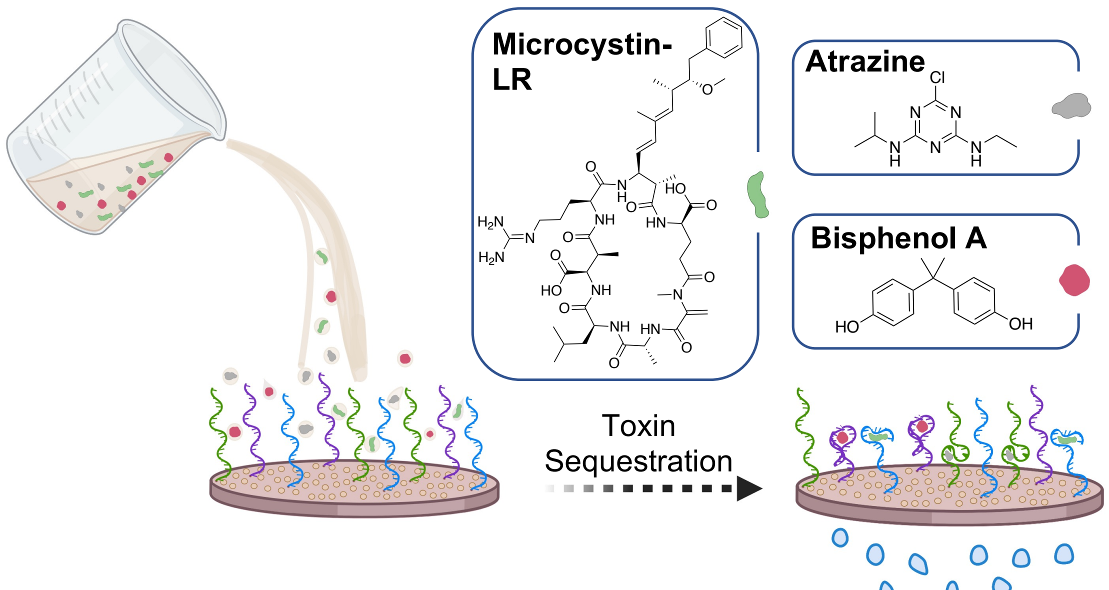
Sequestration and Removal of Multiple Small Molecule Contaminants using an Optimized Aptamer-Based Ultrafiltration System



In this chapter, we enhance the properties of the aptamer-functionalized membrane and investigate the simultaneous removal of multiple small-molecule toxins and contaminants

3.1 Abstract

Small-molecule toxins pose a significant threat to human health and the environment, and their removal is made challenging by their low molecular weight. Aptamers show promise as affinity reagents for binding these toxins, and recently aptamers have been utilized for both sensing and remediation applications. We have found that functionalization of ultrafiltration membranes with aptamers provides a convenient scaffold for toxin sequestration, but our initial efforts in this area were limited by low functionalization efficiencies and the ability to only capture a single target molecule. Herein, we describe detailed optimization of our aptamer-functionalized ultrafiltration membrane system and subsequent use for simultaneous removal of multiple small-molecule toxins. We examine multiple critical components involved in fabricating and functionalizing the membranes, including PEG polymer molecular weight for membrane fabrication, grafting conditions for pMAA attachment, and coupling reagents for aptamer functionalization. This screening enabled us to identify a set of unique conditions in which we were able to achieve high flux, near quantitative yield for DNA attachment, and effective overall depletion of both toxins and bacterial cells. Furthermore, we demonstrate attachment of multiple aptamers and subsequent parallel removal of atrazine, bisphenol A, and microcystin-LR in a



complex lake water matrix. Our rigorous evaluation resulted in depletion of multiple small-molecule toxins and contaminants, demonstrating the potential of aptamer-functionalized membranes as point-of-use decontamination systems.

3.2 Introduction

Aptamers have emerged as promising affinity reagents for use in a wide range of applications,[1-3] owing to their ability to bind to a specific molecule of interest, their high chemical and thermal stability, and the ability to generate aptamers for a wide range of target molecules via *in vitro* selection.[4] To date, aptamers have been primarily used for biomedical applications including drug delivery, therapeutics, clinical diagnostics, imaging, and biomarker discovery.[5-6] However, more recently, they have also seen increasing use for environmental applications such as detection and removal of small-molecule toxins and contaminants in aqueous and biological matrices.[7-9]

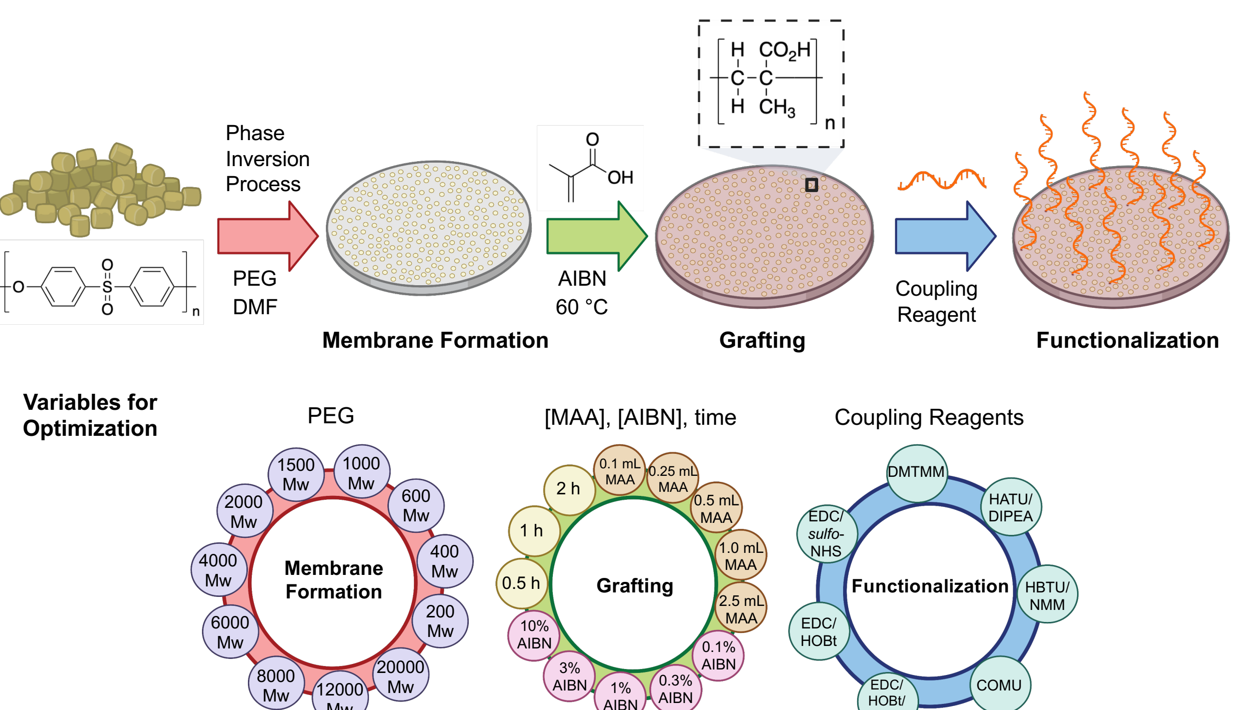
A large number of aptamer-based biosensors have been reported for toxin detection and typically involve coupling of the target binding event to an electrochemical or optical output for quantification.[10-11] Given the ability of aptamers to bind to and sequester molecules of interest, researchers have also explored the use of aptamers in environmental water remediation, though examples of this use remain much more sparse in the literature.[12-13] To achieve sequestration, the aptamer must be immobilized on a solid support, and scaffolds that have been explored include TiO_2 and PLA-PEG nanoparticles, Sepharose beads, and hydrogels.[14-18] While effective, most of these methods require expensive machinery for fabrication or implementation and they can be susceptible to corrosion or biofouling. Additionally, most of these materials have not been shown to be capable of regeneration, limiting them to a single use.[12] Thus, while these technologies are able to remove the molecules of interest, they are limited by challenges with scale-up or time and material costs and are not amenable for point-of-use decontamination in resource limited environments.[19]

With an increase of contaminated water sources worldwide, the sequestration of small-molecule contaminants and toxins has become a priority and unique scaffolds are needed which can effectively sequester and remove these contaminants from an aqueous matrix.[20] Ultrafiltration membranes offer an advantageous scaffold, as they are fabricated from inexpensive materials using facile preparatory techniques and water can pass through them under pressures that can be easily generated using human power.[21] However, given their relatively large pore sizes, they lack the ability to sequester small molecules.[21] To overcome this challenge, we have previously demonstrated that aptamers can be covalently attached to ultrafiltration membranes by grafting poly(methacrylic acid) (pMAA) on the membrane, which introduces a carboxylic acid moiety, which can subsequently be reacted with an amine-modified aptamer.[22] We initially demonstrated the ability of this membrane system to remove Bisphenol A (BPA) from drinking water and environmental water samples. However, most water samples requiring purification contain multiple small-molecule contaminants including pharmaceuticals, pesticides, mycotoxins, and cyanotoxins, as well as small organisms such as bacteria.[23-24] To ensure practicality and efficacy in point-of-use applications, it is critical that an aptamer-based filtration device be able to retain and sequester multiple contaminants as well as small organisms.[25-26]

Herein, we systematically optimize each step of the process for fabrication of our aptamer functionalized membranes, from membrane formation to grafting to aptamer attachment. At each step, we rigorously characterize membrane performance or attachment yield to identify a set of optimized conditions. We also explore the relationship between membrane pore size and removal of bacteria (*Escherichia coli*), demonstrating the capacity to simultaneously sequester small molecules and single-celled organisms. Moreover, we establish the versatility of our membrane system by attaching a combination of different aptamers and demonstrating the ability to sequester multiple small molecules in parallel without impacting depletion efficacy. Together, this research advances the application of aptamer-functionalized membranes as a user-friendly and scalable approach to water purification.

3.3 Results and Discussion

For our initial demonstration of aptamer-functionalized membranes, we adapted standard conditions that had previously been reported in the literature for analogous fabrication and functionalization procedures.[22, 27-28] While this allowed for successful membrane generation, we recognized that significant benefit would be realized by systematically investigating and optimizing each step of the process. As outlined in **Scheme 3.1**, we explore a wide range of conditions for membrane formation, grafting, and functionalization, characterizing function at each step to arrive at an optimized protocol that maximizes both membrane flux and ability to remove small-molecule contaminants and small organisms.



Scheme 3.1: Optimization of key steps in fabrication of aptamer-functionalized membranes. Key parameters that are explored include: (1) molecular weight of PEG additives used in membrane formation (2) concentrations of MAA and AIBN, and reaction time for pMAA grafting (3) coupling reagents used for aptamer functionalization.

3.3.1 Membrane Formation

Membranes are formed from PES using a phase inversion process, and PEG is used as an additive to promote pore formation.[29] Given that pore size impacts flux and other key characteristics, we reasoned that the molecular weight of the PEG was likely to play a substantial role in membrane performance. Thus, we started our optimization process by using PEG having different molecular weights, added to the dope solution when forming the ultrafiltration membranes. As shown in **Figure 3.1**, the use of different molecular weights of this additive changes the internal pore formation and the distribution of macrovoids, therefore modulating water filtration characteristics such as flux and solute rejection. We prioritized water flux, as maintaining high flux is critical to ensuring that the membranes can be operated in a point-of-use setting under human power (**Figure 3.2 a-b**).[19] The data in **Figure 3.1a** demonstrate that there is an increase in flux as the PEG Mw increases from PEG200 until it reaches a plateau around PEG2000-PEG4000, before showing a steady decrease up to PEG20000, which was the largest PEG dopant tested. These data provided insight that membranes formed with PEG dopant in the range of PEG2000–PEG4000 provided optimal flux properties.

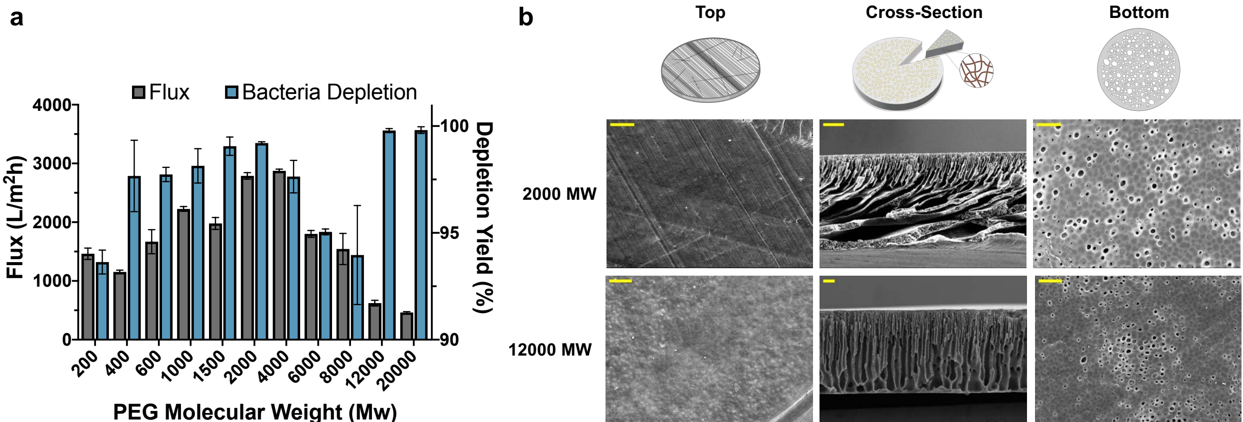


Figure 3.1: (a) Flux and bacteria depletion as a function of molecular weight of PEG dopant. The error bars represent standard deviation from 6 independent trials. **(b)** SEM images of membranes formed using PEG 2000 (top row) and PEG 12000 (bottom row), showing morphology at the top, bottom, and mid cross-section of the membrane. Scale bars are 10 μm for images of top and bottom of membrane and 50 μm for images of membrane cross-section.

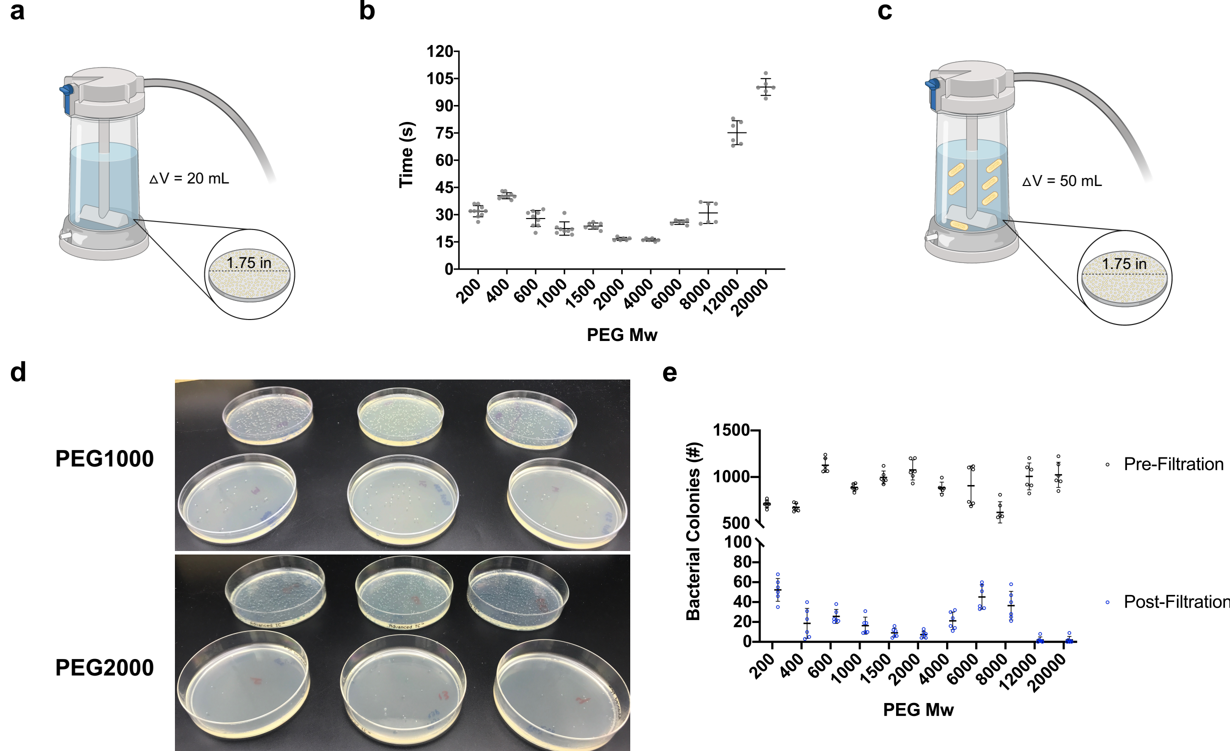


Figure 3.2: **(a)** Flux experiments were performed using a stirred cell filtration apparatus containing a prepared ultrafiltration membrane having a diameter of 1.75 in. **(b)** Time measurements of 20 mL filtrations of distilled water based on different PEG molecular weight dopants used in membrane fabrication. **(c)** *E. coli* depletion using 50 mL of 8000 cells/mL bacteria (*E. coli*) in water. **(d)** Observed colonies before and after filtration with the two representative sample membranes (PEG1000 and PEG2000). **(e)** Differences between bacterial colony count before and after filtration, which were used to assess bacterial depletion yield.

In considering the practical application of these water purification membranes, we recognized that most use would involve mixed matrices that contain both small-molecule contaminants and microorganisms such as bacteria.[30-31] We also recognized that since PEG molecular weight directly impacts pore size, this is the ideal metric to vary in order to optimize bacteria removal. Thus, we screened our membranes formed using various PEG sizes and quantified their ability to deplete *E. coli.* Water spiked with bacterial cells at a concentration of 8000 CFU/mL was filtered through each membrane and samples from before and after filtration were spread on agarose plates, incubated at 37 ^{o}C, and colonies quantified (**Figure 3.2c-e**). While the membranes having the lowest flux (PEG12000 and PEG20000) gave nearly quantitative

bacterial depletion, we were surprised to observe that flux did not directly correlate with bacterial depletion as might be anticipated solely based on pore size (**Figure 3.1a**).

Considering both flux and bacterial removal, we concluded that PEG2000 serves as the best dopant size for preparing our ultrafiltration membranes. However, due to the exceptionally high performance with bacterial depletion and the recognition that other smaller microorganisms may also be present in water samples, we also chose to continue investigating membranes formed using PEG12000, as some sacrifice of flux could be a favorable tradeoff for higher bacterial depletion. Membranes formed using each of these PEG dopant sizes were characterized using SEM (**Figure 3.1b**). The PEG2000 membranes displayed prominent large macrovoids in the cross-section, which was also consistent with the formation of more pores on the bottom of the membrane, and therefore higher flux. In contrast, the PEG12000 membranes show fewer pores, which likely explains the lower flux and higher bacterial depletion.

3.3.2 Membrane Grafting

The next step in our membrane fabrication process is grafting of the PES with pMAA via radical polymerization. The carboxylic acid functional groups on pMAA serve two important purposes, in that they increase the hydrophilicity of the membrane for better use with aqueous matrices[32-33] and provide an attachment point for subsequent functionalization with amine-modified aptamers. Given that maximizing aptamer attachment represents a key desired outcome, we analyzed grafting conditions by monitoring DNA functionalization yield using a constant set of coupling conditions. To optimize grafting, we surveyed a range of methacrylic acid (MAA) volumes (added to 5 mL water for each reaction), 2,2′-azobis(2-methylpropionitrile) AIBN concentrations, and polymerization reaction times with our two previously identified PEG2000 and PEG12000 membranes (**Figure 3.3**). We benchmarked the performance of each membrane against our previously reported conditions (1.5 mL of MAA, 0.3% AIBN, and 1 h of reaction time after MAA addition). We then reacted each membrane with the anti-BPA aptamer[34] using *N*-(3-

dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride/*N*-hydroxysulfosuccinimide sodium salt (EDC/*sulfo*-NHS) as a coupling reagent and quantified aptamer attachment efficiency as previously reported.[22]

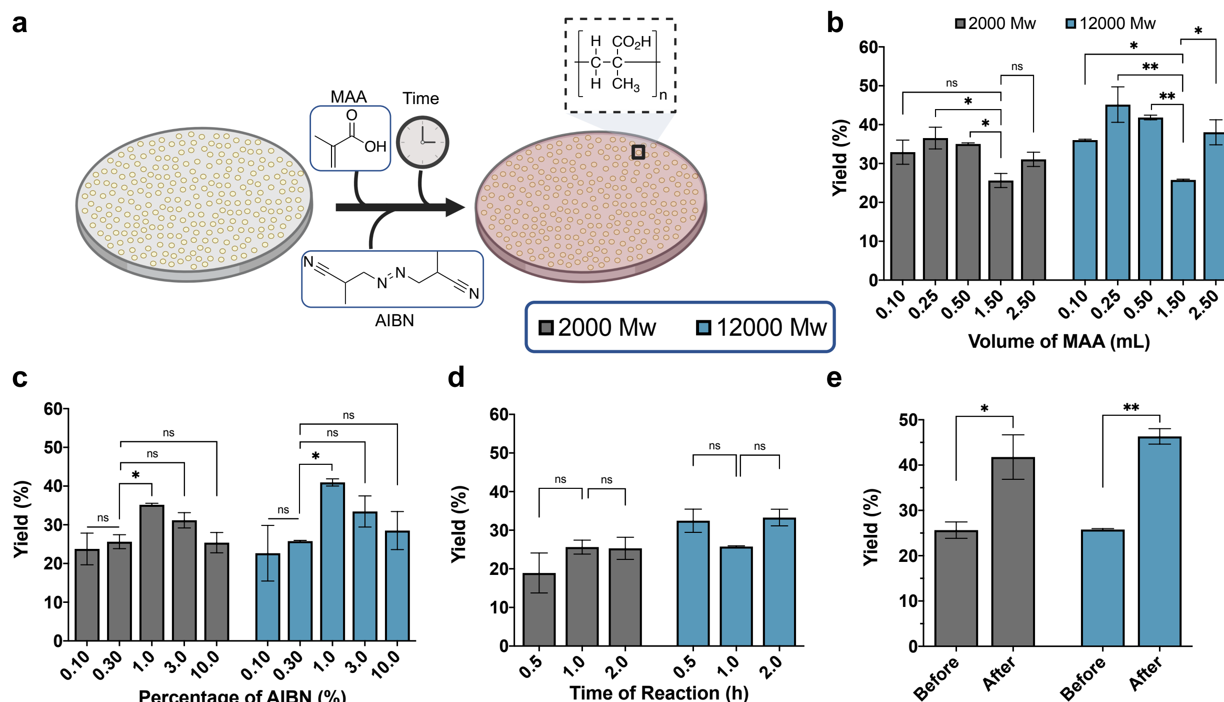


Figure 3.3: (a) Optimization of membrane grafting using membranes formed with PEG2000 and PEG12000. Yield of aptamer attachment resulting from: **(b)** varying volumes of MAA (added to 5 mL water); **(c)** varying concentrations of AIBN initiator; **(d)** varying polymerization reaction time. **(e)** Incorporating all of the optimized conditions results in a significant increase in yield for aptamer attachment. The error bars represent standard deviation of 3 independent trials (*$p < 0.05$, **$p < 0.01$, one-way ANOVA with Tukey's multiple comparison for **(b) – (d)** and two-tailed t-test for **(e)**).

For MAA volume, we observe reproducible impacts on aptamer functionalization, but no distinct relationship between the two variables for either of the membranes (**Figure 3.3b**). Given that the best performance was obtained using 0.25 mL of MAA, we identified this as our volume for use in future experiments. We do note that this condition provides significantly higher attachment compared to the 1.5 mL of MAA used in our initial experiments. In the case of AIBN concentration, we observe that lower amounts of AIBN than used our previously described conditions (0.3%) does not produce a significant difference in functionalization yield. However, we do note a significant functionalization yield increase when AIBN concentration is increased

from 0.3% to 1%, but further increases to 3% and 10% have an opposite effect (**Figure 3.3c**). One potential explanation for these results could be linked to the size of pMAA polymers formed. We hypothesize that increased AIBN should lead to higher numbers of initiation events, and while this could give higher MAA loading, it could also decrease the average pMAA molecular weight if all of the MAA is consumed. While we did not directly study this factor, the size of pMAA may impact functionalization yield, as longer chains will provide greater relief from the steric constraints of the membrane itself. Independent of reasoning for the optimized concentration, we identified 1% AIBN as the optimal condition. As a final parameter, we decided to also vary polymerization reaction time. We varied the reaction time from 0.5-2 h, but no significant differences were observed when increasing or lowering the time, so we decided to maintain our original polymerization time of 1 h (**Figure 3.3d**).

After individually optimizing each reaction parameter, we compared the optimized conditions to those in our previous report and found that our newly proposed grafting conditions increase the yield for aptamer attachment by ~20% for each membrane (**Figure 3.3e**). These new conditions also showed an increase in functionalization yield with aptamers for other small-molecule targets such as atrazine[35], acetamiprid[36-37], and Microcystin-LR (MC-LR)[38] (**Figure 3.4**).

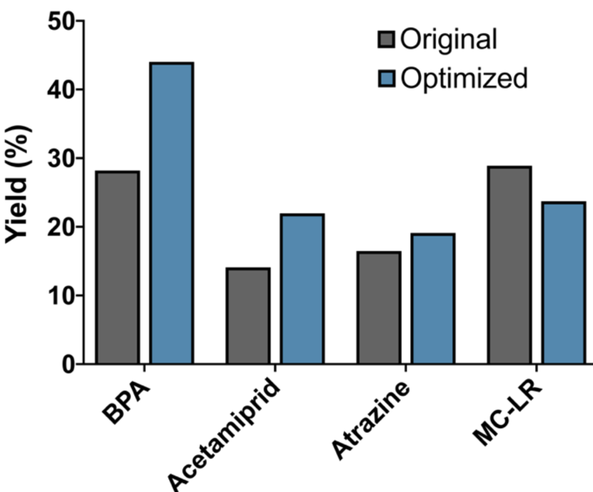


Figure 3.4: Aptamer attachment efficiency using aptamers having different lengths and targets, before and after grafting optimization. Generally, we observed an increase in DNA functionalization yield after grafting optimization.

Thus, our approach is adaptable to aptamers (or other nucleic acids) having varying lengths and secondary structures.

3.3.3 Membrane Functionalization

The DNA aptamer represents the costliest component of the membrane system, and thus optimizing yield for attachment is a critical goal for improving the practicality of scaleup. We were encouraged by the increased yield we were able to obtain through modification of our grafting conditions, and hypothesized that further gains might be realized by also screening reaction conditions for the bioconjugation reaction itself. We tested a variety of common coupling reagents used for amide bond formation[39] and compared these to our previously reported protocol using EDC/*sulfo*-NHS (**Figure 3.5b-c**). We observed that the addition of different catalysts to the EDC

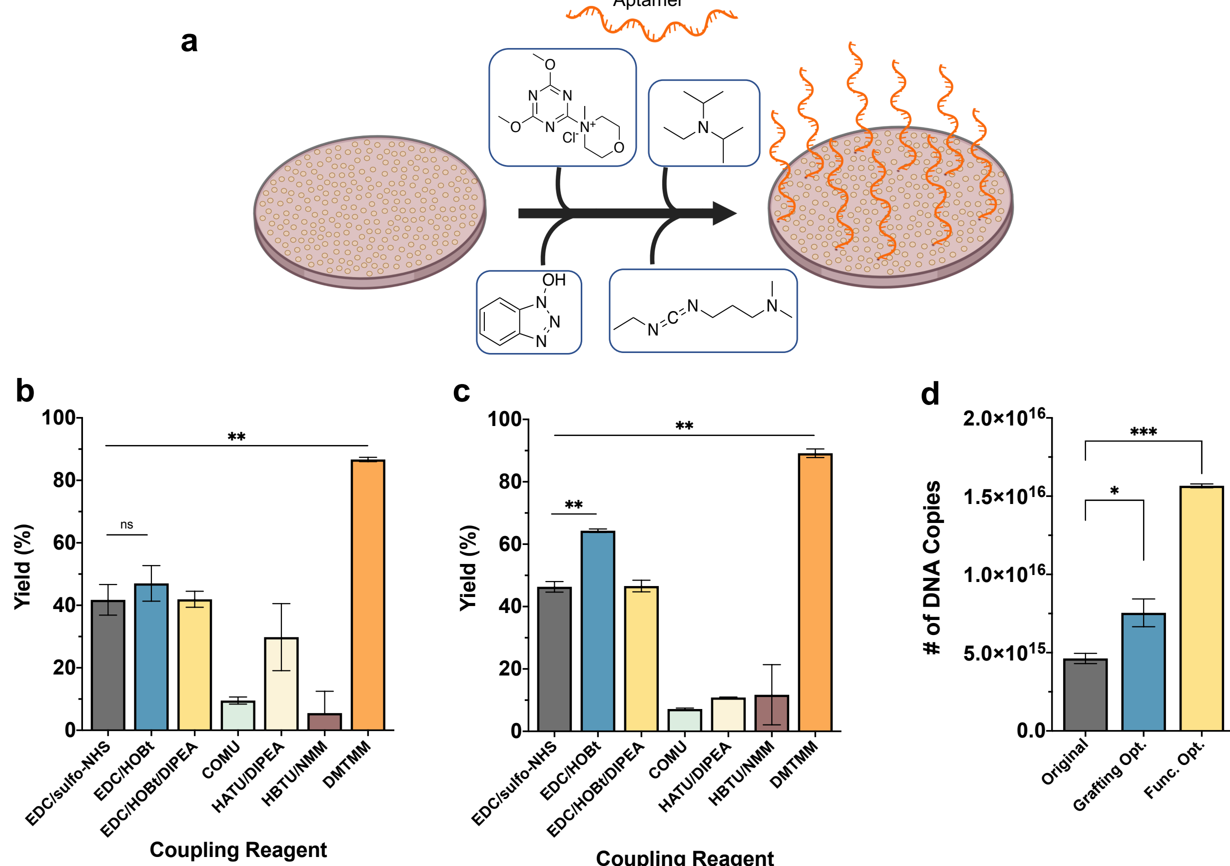


Figure 3.5: (a) Optimization of aptamer functionalization using various coupling reagents. Each coupling condition was tested on membranes generated with **(b)** PEG2000 and **(c)** PEG12000. **(d)** Significant increase in DNA copy number after completion of the comprehensive grafting and functionalization optimization. The error bars represent standard deviation of 3 independent trials (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, two-tailed t-test).

such as 1-hydroxybenzotriazole (HOBt) and *N,N*-diisopropylethylamine (DIPEA) had little effect on yield, with only a small increase observed using HOBt with the PEG12000 membrane. Other coupling reagents such as (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU), 1-[*bis*(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) and N,N,N′,N′-tetramethyl-O-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) show a decrease in yield for both membranes. However, we were very encouraged to observe that 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM), showed outstanding results, with an 87-90% functionalization yield across both membranes. **Figure 3.5d** demonstrates the combined effects of our optimization study, as we are able to more than triple the amount of DNA attached to the membrane. Importantly, with the high yields obtained in our final conditions, very little DNA is wasted in the fabrication process. Moreover, we hypothesize that higher functionalization densities could be obtained by using increased amounts of DNA.

3.3.4 Multi-Analyte Removal

After identifying our optimized conditions for membrane formation (PEG2000 or PEG12000 dopant), membrane grafting (0.25 mL MAA, 1% AIBN, 1 h reaction time), and functionalization (DMTMM), we sought to demonstrate the versatility of the membrane system to be used for depletion of structurally-diverse small-molecule contaminants, or even simultaneous removal of multiple contaminants. First, we created single-aptamer membranes using the aptamers for BPA, atrazine, and MC-LR, which represent a wide range of nucleic acid sizes. Encouragingly, we observed consistent high yields for attachment of each of these aptamers: 87% for BPA (14 nt), 88% for atrazine (32 nt), and 79% for MC-LR (60 nt) (**Figure 3.6a-b**). As a control, we subjected an ungrafted membrane to the same coupling conditions and observed no attachment (**Figure 3.7**).

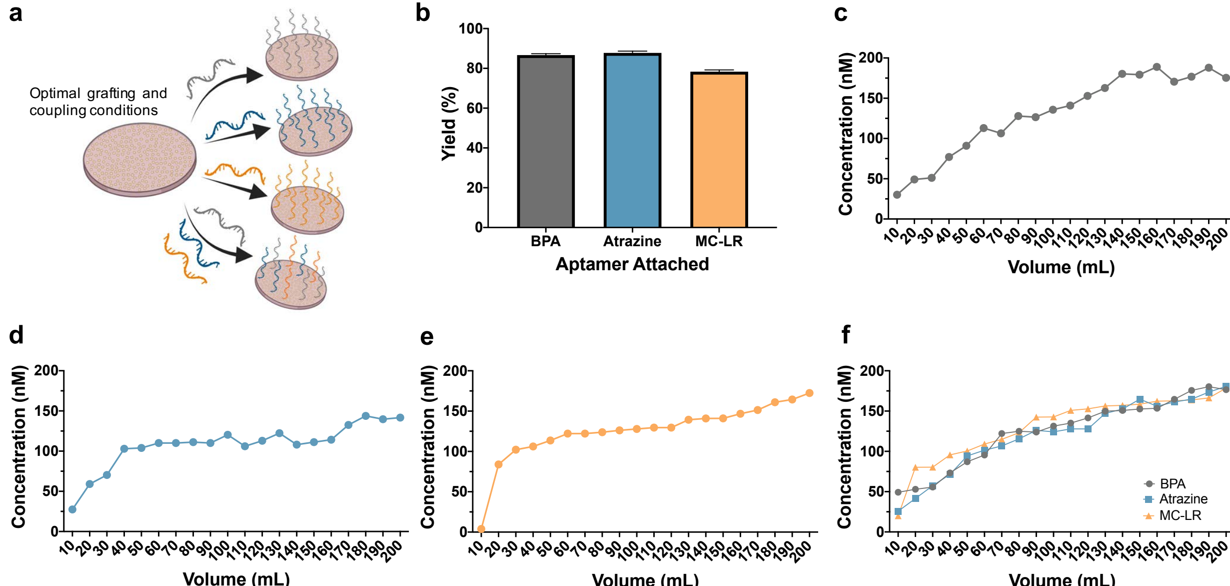


Figure 3.6: (a) Membrane functionalization with aptamers for BPA (blue), Atrazine (red), and Microcystin-LR (green). **(b)** Functionalization efficacy for each aptamer. Error bars represent standard deviation of 3 independent trials. Depletion capacity of membranes functionalized with aptamer for **(c)** BPA only, **(d)** Atrazine only, and **(e)** MC-LR only, using 200 nM spiked Milli-Q water as the feed solution. **(f)** Simultaneous depletion of BPA, atrazine, and MC-LR from a membrane functionalized with all three aptamers.

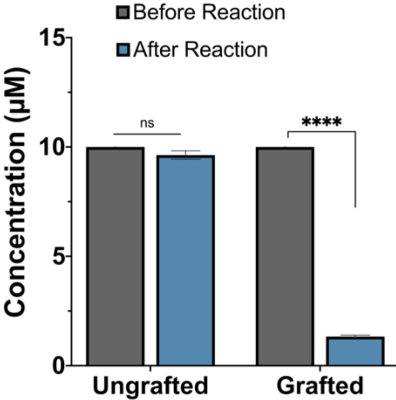


Figure 3.7: Comparison between grafted and ungrafted membrane for functionalization using a Cy3 labeled BPA aptamer. The decrease in concentration is due to the coupling of the oligo to the grafted membrane. The lack of significant change for the ungrafted membrane demonstrates that pMAA is essential for functionalization.

To test depletion efficiency and selectivity for each small-molecule target, water spiked with 200 nM of contaminant was flowed through each membrane, and concentration of each small-molecule in the eluent was quantified by HPLC. As expected, we observed higher depletion for the early fractions, as more aptamer is available for binding, and this is consistent with our earlier report. We were unsure, however, whether all of the aptamers would be capable of depletion with the same efficiency. Thus, we were pleased to observe similar depletion curves for each of the single-aptamer membranes (**Figure 3.6c-e)**. We also found that total ligand binding capacities were similar at 14 nmol for BPA, 18 nmol for atrazine, and 15 nmol for MC-LR.

After demonstrating successful depletion of each contaminant on a single-aptamer membrane, we focused on exploring whether we could attach all three different aptamers to a single membrane without compromising functionalization yield or depletion capacity. We observed similar functionalization yields as had been achieved for single-aptamer membranes (**Figure 3.8**), which is especially encouraging as it demonstrates that our overall loading of DNA on the membrane can be increased far beyond the levels used in our initial experiments. We then subjected the membrane to filtration with water spiked with 200 nM of each of the three

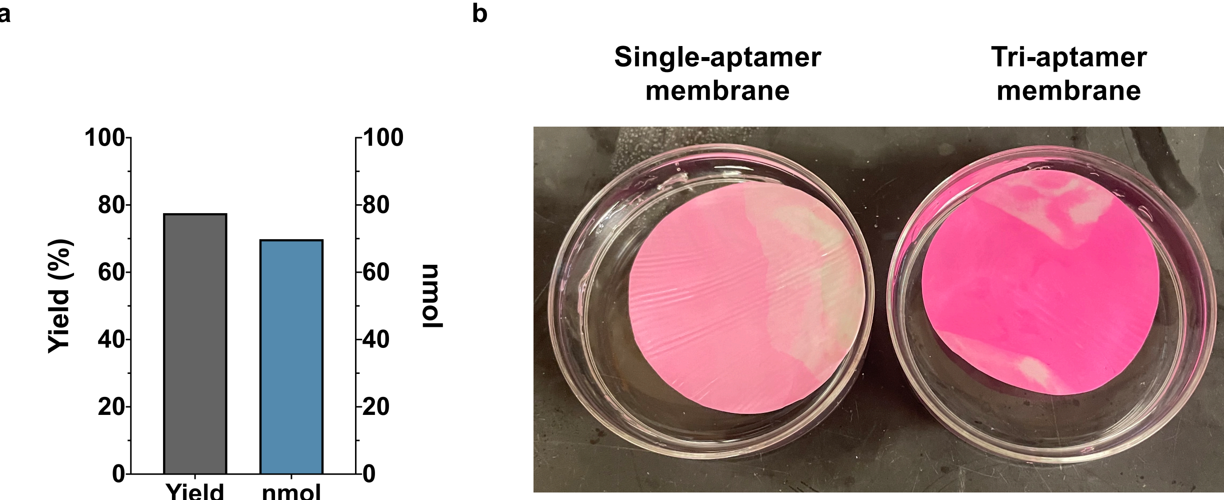


Figure 3.8: (a) Yield for attachment of 3 different aptamers (BPA, MC-LR, and atrazine at 10 μM each) to a single ultrafiltration membrane. **(b)** Qualitative assessment where a colorimetric difference is observed between a single-aptamer functionalized membrane (MC-LR) and a tri-aptamer functionalized membrane.

contaminants, and observed that the depletion capacity of the tri-aptamer membrane was similar to that of each of the single-aptamer membranes, demonstrating that increased DNA loading and the presence of multiple aptamers and targets does not significantly impact performance (**Figure 3.6f, Figure 3.9**).

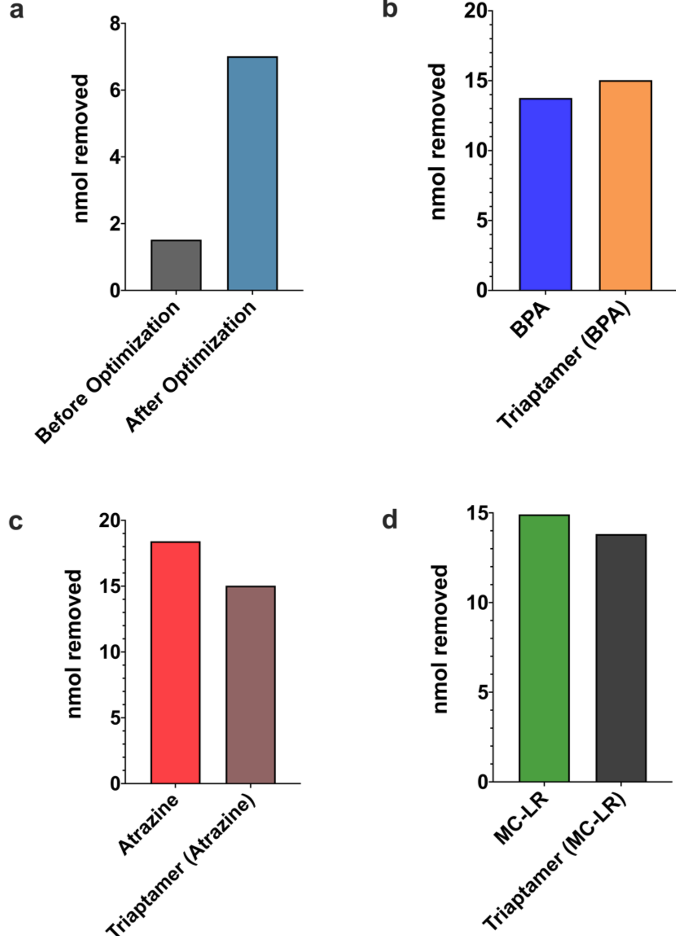


Figure 3.9: (a) Comparison of nmol of BPA removed before reaching EPA intake limit. A five-fold increase in breakthrough volume is observed for optimized membrane. Differences in depletion (nmol of contaminant) between a single-aptamer and a tri-aptamer functionalized membrane based on the different small-molecules tested **(b)** BPA, **(c)** atrazine, and **(d)** MC-LR. No major differences in removal yields are observed for single- vs tri-functionalized membranes.

Most importantly, comparing the depletion capacities from our previous study with those of our optimized system revealed a 5-fold increase in capacity for membranes of the same size, which is largely attributable to our increased yield for functionalization. This is important, as

increasing capacity allows a membrane to filter a greater volume of water before breaking through the allowable limits for each toxin in drinking water.

3.3.5 Application in Environmental samples

To demonstrate the practical utility of aptamer-functionalized membranes in environmental samples, we tested our system in lake water from a local source. We obtained lake water from Chandler Lake-Lullwater Preserve at Emory University and evaluated the presence of the contaminants that we were interested in removing. After finding that these contaminants were not present at detectable levels in this water source, we generated a contrived environmental sample by spiking the water with the same concentration of contaminants used in our previous experiments (**Figure 3.10**). As a control, we filtered this sample through a grafted membrane with no aptamers attached, and we observed that the concentration of the different contaminants remains constant, demonstrating that depletion does not arise from non-specific adsorption of the small molecules (**Figure 3.10a**). We then repeated the filtration using a tri-functionalized membrane having BPA, atrazine, and MC-LR aptamers, and these samples showed depletion of their respective small molecules in a similar manner to that observed for a tap water sample (**Figure 3.10b** and **Tables 3.1-3.3**). This further demonstrates the versatility and utility of the

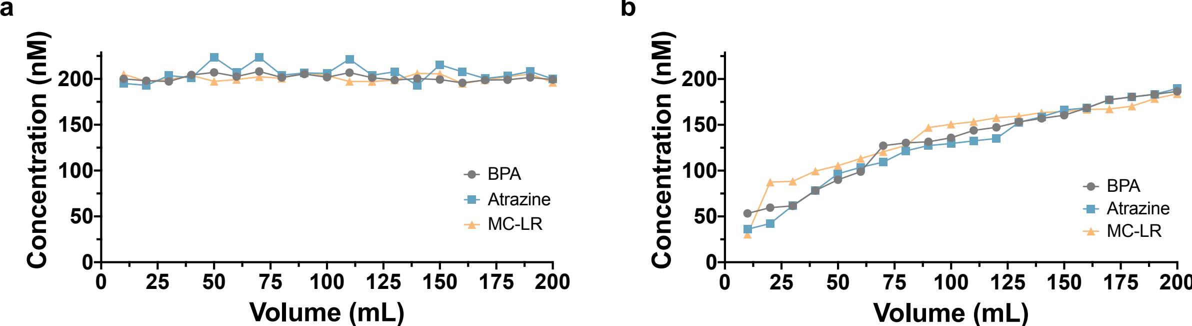


Figure 3.10: Depletion of small-molecule contaminants using a heterogenous lake water sample containing spiked contaminants. **(a)** Depletion capacity using a grafted (non-functionalized) membrane, showing no change in concentration. **(b)** Depletion capacity of lake water using a membrane functionalized with multiple aptamers.

purification system we have created, as it is able to simultaneously remove multiple small-molecule toxins from a complex environmental sample.

Table 3.1: Depletion of tri-aptamer functionalized membrane for the small-molecule contaminant BPA in lake water.

Volume (mL)	Concentration (nM)	DNA in Supernatant (nmol)	DNA Removed (nmol)
10	53.39	0.53	1.47
20	59.62	0.60	1.40
30	61.48	0.61	1.39
40	78.42	0.78	1.22
50	90.06	0.90	1.10
60	98.85	0.99	1.01
70	127.32	1.27	0.73
80	130.39	1.30	0.70
90	131.51	1.32	0.68
100	135.82	1.36	0.64
120	143.99	1.44	0.56
130	147.27	1.47	0.53
140	153.14	1.53	0.47
150	157.06	1.57	0.43
160	160.51	1.61	0.39
170	168.43	1.68	0.32
180	177.48	1.77	0.23
190	180.58	1.81	0.19
200	183.26	1.83	0.17

Table 3.2: Depletion of tri-aptamer functionalized membrane for the small-molecule contaminant atrazine in lake water.

Volume (mL)	Concentration (nM)	DNA in Supernatant (nmol)	DNA Removed (nmol)
10	35.99	0.36	1.64
20	42.27	0.42	1.58
30	61.79	0.62	1.38
40	78.51	0.79	1.21
50	96.58	0.97	1.03
60	103.65	1.04	0.96
70	109.26	1.09	0.91
80	121.57	1.22	0.78
90	127.39	1.27	0.73
100	129.62	1.30	0.70
120	132.48	1.32	0.68
130	135.10	1.35	0.65
140	152.66	1.53	0.47
150	158.85	1.59	0.41
160	166.32	1.66	0.34
170	168.43	1.68	0.32
180	177.48	1.77	0.23
190	180.37	1.80	0.20
200	183.41	1.83	0.17

Table 3.3: Depletion of tri-aptamer functionalized membrane for the small-molecule contaminant MC-LR in lake water.

Volume (mL)	Concentration (nM)	DNA in Supernatant (nmol)	DNA Removed (nmol)
10	30.43	0.30	1.70
20	87.48	0.87	1.13
30	88.43	0.88	1.12
40	99.48	0.99	1.01
50	105.48	1.05	0.95
60	113.10	1.13	0.87
70	120.62	1.21	0.79
80	127.48	1.27	0.73
90	147.06	1.47	0.53
100	150.62	1.51	0.49
120	153.39	1.53	0.47
130	157.62	1.58	0.42
140	159.48	1.59	0.41
150	163.29	1.63	0.37
160	164.66	1.65	0.35
170	166.85	1.67	0.33
180	167.30	1.67	0.33
190	170.39	1.70	0.30
200	178.66	1.79	0.21

3.4 Experimental Section

3.4.1 Materials

Chemicals including the polymers, coupling reagents, molecular contaminants were obtained from Sigma Aldrich Corporation (St. Louis, MO, USA) and were used without further purification. Chemicals include poly(oxy-1,4-phenylenesulfonyl-1,4-phenylene) (PES), methacrylic acid (MAA), poly(ethylene glycol)- various molecular weights (PEG), 2,2′-azobis(2-methylpropionitrile) (AIBN), *N,N*-dimethylformamide (DMF), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysulfosuccinimide sodium salt (*sulfo*-NHS), (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU), 1-[*bis*(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxidhexafluorophosphate (HATU), 3-hydroxytriazolo[4,5-b]pyridine (HOAt), 1-hydroxybenzotriazole (HOBt), *N,N*-diisopropylethylamine (DIPEA), N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), 4-methylmorpholine (NMM), 4-(4,6-

dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM), bisphenol A (BPA), mMicrocystin-LR (MC-LR), and atrazine. DNA sequences (**Table 3.4-3.5**) were custom-synthesized from University of Utah DNA/Peptide Synthesis Core (Salt Lake City, UT, USA) and from Integrated DNA Technologies (Coralville, IA, USA). Membranes were crafted using a Elcometer® 3580/4 casting knife film applicator. Membrane flux experiments were accomplished using an Amicon® Stirred Cell 50 mL (UFSC05001) from Millipore-Sigma (Burlington, MA, USA). Colonies were counted using a colony counter pen manufactured by VWR® (Radnor, PA, USA).

Table 3.4: List of DNA sequences used in this study.

Name	Sequence
BPA Aptamer	5'-GGATAGCGGGTTCC-3'
Atrazine Aptamer	5'-TACTGTTTGCACTGGCGGATTTAGCCAGTCAGTG-3'
M-LR Aptamer	5'GGCGCCAAACAGGACCACCATGACAATTACCCATACCACCTCATTATG CCCCATCTCCGC-3'
Acetamiprid Aptamer	5'- CTGACACCATATTATGAAGA-3'
BPA Aptamer (FAM)	5'-/FAM/-GGATAGCGGGTTCC-3'
Atrazine Aptamer (FAM)	5'-/FAM/-TACTGTTTGCACTGGCGGATTTAGCCAGTCAGTG-3'
MC-LR Aptamer (FAM)	5'/FAM/GGCGCCAAACAGGACCACCATGACAATTACCCATACCACCTCATT ATGCCCCATCTCCGC-3'
Acetamiprid Aptamer (FAM)	5'-/FAM/-CTGACACCATATTATGAAGA-3'
BPA Aptamer (NH_2)	5'-GGATAGCGGGTTCC-NH_2-3'
Atrazine Aptamer (NH_2)	5'-TACTGTTTGCACTGGCGGATTTAGCCAGTCAGTG-NH_2-3'
MC-LR Aptamer (NH_2)	5'GGCGCCAAACAGGACCACCATGACAATTACCCATACCACCTCATTATG CCCCATCTCCGC-NH_2-3'
Atrazine Aptamer (Cy3)	5'-/Cy3/-TACTGTTTGCACTGGCGGATTTAGCCAGTCAGTG-3'
MC-LR Aptamer (Cy3)	5'/Cy3/GGCGCCAAACAGGACCACCATGACAATTACCCATACCACCTCATT ATGCCCCATCTCCGC-3'
Acetamiprid Aptamer (Cy3)	5'-/Cy3/-CTGACACCATATTATGAAGA-3'

Table 3.5: Chemical structures of DNA modifications used in this study.

Modification	Name	Chemical Structure
FAM	6-Fluorescein	HO, O, OH, O, O, O, HN
Cy3	Cyanine-3	N^+, N, HO, O, O–P, O^-
NH_2	3'-Amino-Modifier C7 CPG 1000	O, O=P–O, OH, NH_2, HO

3.4.2 Fabrication of ultrafiltration membranes

In a closed glass container, PES pellets (2.0 g, 15% w/w), PEG (1.28 g, 10% w/w), and DMF (10 mL, 75% w/w) were mixed at room temperature. This solution was agitated using a nutating mixer until full homogenization was achieved. This dope solution was divided into two equivalent portions and each portion was spread on a 20 cm x 20 cm glass plate using a casting knife film applicator at a width of 500 μm. The glass was then submerged in a tub containing deionized water, causing precipitation of the film and thus formation of the membrane. The membrane remained in the water bath until it detached from the glass. The rough edges were excised, and the membrane was washed with copious amounts of water for 12 h to remove any non-precipitated content. Circular membranes were produced using a stainless-steel round cutter with a size of 1.75 in, followed by trimming of the excess membrane with scissors.

3.4.3 Grafting of ultrafiltration membranes

The circular membrane was combined with 20 mL of degassed water (using N_2) in an appropriate glass vessel. Radicals were formed by the addition of differing amounts of AIBN under a N_2 atmosphere at a temperature of 60 °C. The membrane and initiator were reacted for 20 min on a nutating mixer at 500 rpm. MAA (various amounts) was added dropwise to the reaction mixture to prevent self-polymerization. The reaction mixture was diluted with 5 mL of degassed water and reacted for 1 h at 60 °C. The membrane was removed from the reaction mixture and stored in 50 mL of fresh water. Unbound pMAA was removed by washing with NaOH (1 N, 3 x 50 mL) and water (3 x 50 mL) for 7 min each. The washes followed the alternating sequence: NaOH → H_2O → NaOH → H_2O → NaOH → H_2O. Grafting yield was determined by functionalizing a fluorophore labeled antisense-BPA aptamer (10 µM) using the initial coupling conditions (EDC/*sulfo*-NHS), to verify how much aptamer was attached to the grafted membrane.

3.4.4 Functionalizing grafted membranes

The circular membranes (with optimized grafted conditions) were activated with different coupling reagents with concentrations ranging from 60 – 300 mM for 20 min. (10 min on each side of the membrane). FAM-labeled or Cy3-labeled amine-modified DNA (10 µM) was prepared for membrane functionalization and attachment quantification by diluting the DNA in MOPS buffer (1 M, 0.5 N NaCl, pH 8.5). For initial characterization, a small (50 µL) aliquot was removed from the solution. To ensure thorough functionalization, the membrane was added to the DNA solution and shaken in the nutating mixer for 24 h. The membranes were washed with MOPS buffer (3 x 10 mL) and water (3 x 10 mL) to remove any unbound DNA and further stored in fresh water until further usage. Yield was determined by the change in concentration of the fluorophore labeled aptamer in the supernatant before and after reaction. DNA copy number was estimated from the nanomoles of aptamer that were conjugated to the membrane.

3.4.5 Scanning Electron Microscopy

Circular ultrafiltration membranes were cut into 0.5 x 0.5 cm squares and coated with 20 nm of AuPd in a Denton Desktop II sputter coater. Cross-sections were obtained by submerging the membrane in liquid nitrogen for 1 min and then tearing using tweezers to obtain a clean cross-section cut. SEM (Topcon Ds 130f in high vacuum mode) was used to visualize different morphological features of the membrane samples. Images were analyzed using ImageJ.

3.4.6 Flux Experiments

The membranes were compressed using the stirred-cell filtration apparatus for 10 min at a pressure of 2.5 bar with N_2. Readings were measured at 1 bar. The time required for the water volume to decrease from V_1= 40 mL to V_2= 20 mL (ΔV= 20 mL) was measured. Flux (J) was calculated as shown below in Equation 1 and reported as L/m^{2}h. Q_p is the permeate flow (L/h) and A_{system} is the surface area of the membrane in m^2.

Equation 3.1: Calculation of flux

$$J = \frac{Q_p}{A_{system}}$$

3.4.7 Bacterial Depletion

Overnight cultures of *Escherichia coli (E. coli)* were started in Luria-Bertani (LB) broth and grown at 37 ºC shaking at 200 RPM. Exponential phase cultures (OD_{600} = 0.3 - 0.6) were then used to create solutions of 8000 cells/mL in water. Water (50 mL) spiked with bacteria was filtered through an ultrafiltration membrane using the stirred-cell filtration apparatus. Aliquots of solution, taken before and after filtration, were diluted 10- and 100-fold and were streaked in separate agar plates (100 µL) and were incubated overnight at 37 ºC. Colonies were counted by hand using a colony counter pen to assess the number of colonies formed before and after filtration to determine the percentage of bacteria depletion.

3.4.8 Small-molecule depletion

Depletion of small-molecule contaminants using the functionalized membrane was investigated by filtering a spiked water sample (200 nM of BPA, MC-LR, atrazine, or all three) as the feed solution. This was performed using a stirred cell apparatus by compressing the membranes for 10 min at a pressure of 2.5 bar with N_2. The system was filtered at 1 bar and the permeate (10 mL fractions) was collected and 1.5 mL of each fraction was concentrated using vacuum centrifugation. The samples were analyzed as previously described.[22] In short, using HPLC, a calibration curve ranging from 0 nM-200 nM was obtained and concentrations of the different fractions were determined by comparing peak area to that from a calibration curve.

3.5 Conclusions

In summary, we describe here the optimization of membrane fabrication, grafting, and aptamer functionalization to maximize aptamer loading and thus toxin depletion of ultrafiltration membranes. We observed that PEG2000 is an ideal pore forming agent in membrane formation as it provides membranes having high flux and the ability to remove most bacterial cells. Our grafting conditions screen showed that increasing the concentration of the radical initiator and decreasing the concentration of MAA relative to our previous conditions provided improved aptamer attachment yields. We were also able to identify a high-yielding coupling reagent (DMTMM) that aids in the amide bond formation reaction to give near quantitative DNA functionalization. With these enhanced parameters, we were able to reliably attach aptamers having varying sizes and secondary structures and achieve depletion of structurally diverse contaminants. Furthermore, we were able to attach three aptamers to the membrane, overall tripling the DNA loading and enabling simultaneous removal of three contaminants without a significant decrease in depletion efficiency.

Given the reported improvements in functionalization yield, we hypothesize that the membranes could be scaled to create personal use filters able to purify the equivalent amount of drinking water for one person for one day. And, we have reported previously that the membrane can be easily regenerated using a small volume of warm water to reversibly denature the aptamers.[22] Additionally, the flexibility to work with multiple aptamer-analyte systems will allow for the creation of membranes that are customized to local water purification needs. Together, aptamer-functionalized ultrafiltration membranes hold significant promise for point-of-use decontamination of water, offering potential to advance environmental and human health.

3.6 References

1. Liu, L. S.; Wang, F.; Ge, Y.; Lo, P. K., Recent Developments in Aptasensors for Diagnostic Applications. *ACS Applied Materials & Interfaces* **2021,** *13* (8), 9329-9358.

2. Ni, S.; Zhuo, Z.; Pan, Y.; Yu, Y.; Li, F.; Liu, J.; Wang, L.; Wu, X.; Li, D.; Wan, Y.; Zhang, L.; Yang, Z.; Zhang, B.-T.; Lu, A.; Zhang, G., Recent Progress in Aptamer Discoveries and Modifications for Therapeutic Applications. *ACS Applied Materials & Interfaces* **2021,** *13* (8), 9500-9519.

3. Fan, C.; Wang, S.; Schanze, K.; Fernandez, L. E., Materials Applications of Aptamers. *ACS Applied Materials & Interfaces* **2021,** *13* (8), 9289-9290.

4. Ruscito, A.; DeRosa, M. C., Small-Molecule Binding Aptamers: Selection Strategies, Characterization, and Applications. *Frontiers in Chemistry* **2016,** *4* (14).

5. Afrasiabi, S.; Pourhajibagher, M.; Raoofian, R.; Tabarzad, M.; Bahador, A., Therapeutic applications of nucleic acid aptamers in microbial infections. *Journal of Biomedical Science* **2020,** *27* (1), 6.

6. Xie, S.; Ai, L.; Cui, C.; Fu, T.; Cheng, X.; Qu, F.; Tan, W., Functional Aptamer-Embedded Nanomaterials for Diagnostics and Therapeutics. *ACS Applied Materials & Interfaces* **2021,** *13* (8), 9542-9560.

7. Saad, M.; Faucher, S. P., Aptamers and Aptamer-Coupled Biosensors to Detect Water-Borne Pathogens. *Frontiers in Microbiology* **2021,** *12* (304).

8. Schmitz, F. R. W.; Valério, A.; de Oliveira, D.; Hotza, D., An overview and future prospects on aptamers for food safety. *Applied Microbiology and Biotechnology* **2020,** *104* (16), 6929-6939.

9. McConnell, E. M.; Nguyen, J.; Li, Y., Aptamer-Based Biosensors for Environmental Monitoring. *Frontiers in Chemistry* **2020,** *8* (434).

10. Arvand, M.; Mirroshandel, A. A., An efficient fluorescence resonance energy transfer system from quantum dots to graphene oxide nano sheets: Application in a photoluminescence aptasensing probe for the sensitive detection of diazinon. *Food Chemistry* **2019,** *280*, 115-122.

11. Wei, X.; Zhou, W.; Sanjay, S. T.; Zhang, J.; Jin, Q.; Xu, F.; Dominguez, D. C.; Li, X., Multiplexed Instrument-Free Bar-Chart SpinChip Integrated with Nanoparticle-Mediated Magnetic Aptasensors for Visual Quantitative Detection of Multiple Pathogens. *Analytical Chemistry* **2018,** *90* (16), 9888-9896.

12. Bilibana, M. P.; Citartan, M.; Yeoh, T. S.; Rozhdestvensky, T. S.; Tang, T.-H., Aptamers as the Agent in Decontamination Assays (Apta-Decontamination Assays): From the Environment to the Potential Application In Vivo. *J Nucleic Acids* **2017,** *2017*, 3712070-3712070.

13. Schax, E.; Lönne, M.; Scheper, T.; Belkin, S.; Walter, J.-G., Aptamer-based depletion of small molecular contaminants: A case study using ochratoxin A. *Biotechnology and Bioprocess Engineering* **2015,** *20* (6), 1016-1025.

14. Chen, B.; Ye, Q.; Zhou, K.; Wang, Y., Adsorption and separation of HCV particles by novel affinity aptamer-functionalized adsorbents. *Journal of Chromatography B* **2016,** *1017-1018*, 174-181.

15. Hu, X.; Tulsieram, K. L.; Zhou, Q.; Mu, L.; Wen, J., Polymeric nanoparticle–aptamer bioconjugates can diminish the toxicity of mercury in vivo. *Toxicology Letters* **2012,** *208* (1), 69-74.

16. Song, M. Y.; Jurng, J.; Park, Y.-K.; Kim, B. C., An aptamer cocktail-functionalized photocatalyst with enhanced antibacterial efficiency towards target bacteria. *Journal of Hazardous Materials* **2016,** *318*, 247-254.

17. Hu, X.; Mu, L.; Zhou, Q.; Wen, J.; Pawliszyn, J., ssDNA Aptamer-Based Column for Simultaneous Removal of Nanogram Per Liter Level of Illicit and Analgesic Pharmaceuticals in Drinking Water. *Environmental Science & Technology* **2011,** *45* (11), 4890-4895.

18. Wang, J.; Shen, H.; Hu, X.; Li, Y.; Li, Z.; Xu, J.; Song, X.; Zeng, H.; Yuan, Q., A Targeted "Capture" and "Removal" Scavenger toward Multiple Pollutants for Water Remediation based on Molecular Recognition. *Adv Sci (Weinh)* **2016,** *3* (3), 1500289.

19. Pooi, C. K.; Ng, H. Y., Review of low-cost point-of-use water treatment systems for developing communities. *npj Clean Water* **2018,** *1* (1), 11.

20. Obotey Ezugbe, E.; Rathilal, S., Membrane Technologies in Wastewater Treatment: A Review. *Membranes (Basel)* **2020,** *10* (5), 89.

21. Lee, A.; Elam, J. W.; Darling, S. B., Membrane materials for water purification: design, development, and application. *Environmental Science: Water Research & Technology* **2016,** *2* (1), 17-42.

22. Romero-Reyes, M. A.; Heemstra, J. M., Small-Molecule Sequestration Using Aptamer-Functionalized Membranes. *ACS Materials Letters* **2019,** *1* (5), 568-572.

23. Rojas, S.; Horcajada, P., Metal–Organic Frameworks for the Removal of Emerging Organic Contaminants in Water. *Chemical Reviews* **2020,** *120* (16), 8378-8415.

24. Rathi, B. S.; Kumar, P. S., Application of adsorption process for effective removal of emerging contaminants from water and wastewater. *Environmental Pollution* **2021,** *280*, 116995.

25. Brown, K. W.; Gessesse, B.; Butler, L. J.; MacIntosh, D. L., Potential Effectiveness of Point-of-Use Filtration to Address Risks to Drinking Water in the United States. *Environmental Health Insights* **2017,** *11*, 1178630217746997.

26. Yoo, H.; Jo, H.; Oh, S. S., Detection and beyond: challenges and advances in aptamer-based biosensors. *Materials Advances* **2020,** *1* (8), 2663-2687.

27. Li, Y.; Gabriele, E.; Samain, F.; Favalli, N.; Sladojevich, F.; Scheuermann, J.; Neri, D., Optimized Reaction Conditions for Amide Bond Formation in DNA-Encoded Combinatorial Libraries. *ACS Combinatorial Science* **2016,** *18* (8), 438-443.

28. Shi, Q.; Su, Y.; Ning, X.; Chen, W.; Peng, J.; Jiang, Z., Graft polymerization of methacrylic acid onto polyethersulfone for potential pH-responsive membrane materials. *Journal of Membrane Science* **2010,** *347* (1), 62-68.

29. Ma, Y.; Shi, F.; Ma, J.; Wu, M.; Zhang, J.; Gao, C., Effect of PEG additive on the morphology and performance of polysulfone ultrafiltration membranes. *Desalination* **2011,** *272* (1), 51-58.

30. Xu, H.; Guo, L., Molecular size-dependent abundance and composition of dissolved organic matter in river, lake and sea waters. *Water Research* **2017,** *117*, 115-126.

31. Zhang, H.; Wang, Y.; Chen, S.; Zhao, Z.; Feng, J.; Zhang, Z.; Lu, K.; Jia, J., Water Bacterial and Fungal Community Compositions Associated with Urban Lakes, Xi'an, China. *International Journal of Environmental Research and Public Health* **2018,** *15* (3).

32. Yang, B.; Yang, X.; Liu, B.; Chen, Z.; Chen, C.; Liang, S.; Chu, L.-Y.; Crittenden, J., PVDF blended PVDF-g-PMAA pH-responsive membrane: Effect of additives and solvents on membrane properties and performance. *Journal of Membrane Science* **2017,** *541*, 558-566.

33. Zheng, C.; Zhou, Y.; Zhang, H., Facile Preparation of Well-Defined Uniform Hydrophilic Hairy Hollow Functional Polymer Micro- and Nanoparticles. *ACS Applied Polymer Materials* **2020,** *2* (2), 220-233.

34. Lee, E.-H.; Lim, H. J.; Lee, S.-D.; Son, A., Highly Sensitive Detection of Bisphenol A by NanoAptamer Assay with Truncated Aptamer. *ACS Applied Materials & Interfaces* **2017,** *9* (17), 14889-14898.

35. Williams, R. M.; Crihfield, C. L.; Gattu, S.; Holland, L. A.; Sooter, L. J., In Vitro Selection of a Single-Stranded DNA Molecular Recognition Element against Atrazine. *International Journal of Molecular Sciences* **2014,** *15* (8), 14332-14347.

36. Shi, H.; Zhao, G.; Liu, M.; Fan, L.; Cao, T., Aptamer-based colorimetric sensing of acetamiprid in soil samples: Sensitivity, selectivity and mechanism. *Journal of Hazardous Materials* **2013,** *260*, 754-761.

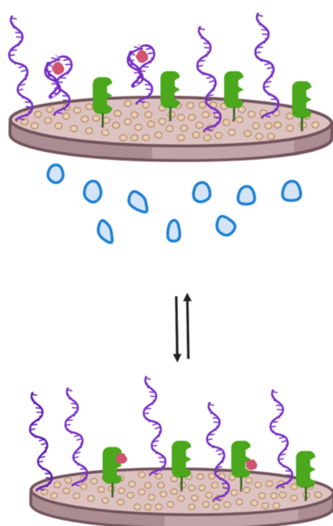
37. He, J.; Liu, Y.; Fan, M.; Liu, X., Isolation and Identification of the DNA Aptamer Target to Acetamiprid. *Journal of Agricultural and Food Chemistry* **2011,** *59* (5), 1582-1586.

38. Ng, A.; Chinnappan, R.; Eissa, S.; Liu, H.; Tlili, C.; Zourob, M., Selection, Characterization, and Biosensing Application of High Affinity Congener-Specific Microcystin-Targeting Aptamers. *Environmental Science & Technology* **2012,** *46* (19), 10697-10703.

39. Valeur, E.; Bradley, M., Amide bond formation: beyond the myth of coupling reagents. *Chemical Society Reviews* **2009,** *38* (2), 606-631.

Chapter 4

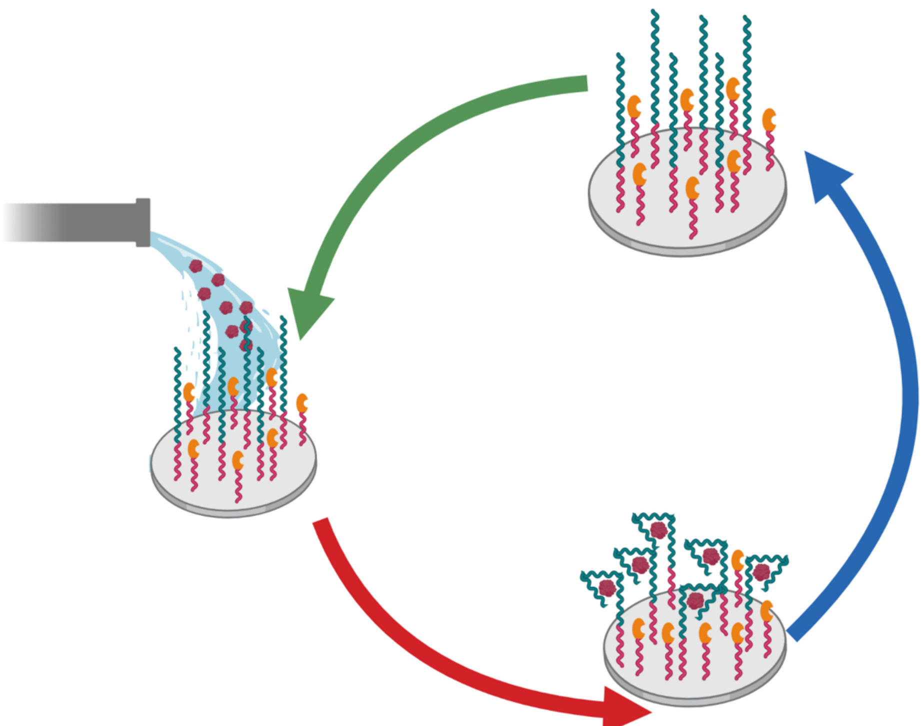
Application of Aptamer-Functionalized Membranes: Sequestration and Degradation of Small Molecules



In this chapter, we envision and study the different applications that aptamer-functionalized membranes can provide.

4.1 Abstract

Synthetic membranes have been used for a variety of applications, but their main purpose has been identified and focused on the removal of contaminants from water. Their characteristic properties had made them ideal to act as a physical barrier for the removal of molecules. Aptamer-functionalized membranes have filled in the gap for the removal of small-molecule contaminants as well as macromolecules. Because of the inherent characteristics of both, aptamers and membranes, the applications go beyond the use of decontamination of water. Because of the durability of the membrane, it can be used as a scaffold to not only attach aptamers, but also enzymes of interest that are able to remove this molecule. This allows for an efficient re-generation of the membrane and continued use for water decontamination. All the research presented in this chapter expands upon the initial application of aptamer-functionalized membranes, opening a research area for the use of aptamers for the removal of small-molecules in an easy and inexpensive way for point-of-use application.



4.2 Introduction

Aptamers are nucleic acid sequences that can be evolved to bind to a wide range of small-molecule targets with high affinity and specificity.[1] Aptamers are generated by Systematic Evolution of Ligands by Exponential Enrichment (SELEX), a process in which a library having a large (1012-1014) number of sequences is incubated with a ligand and sequences that show the desired binding activity are enriched over multiple rounds.[2-4] Aptamer sequences generated by SELEX are typically 60-100 nt in length but can be minimized post-selection to provide significantly shorter sequence lengths, which facilitates their synthesis and use in downstream applications.[5] They are generated through chemical synthesis, allowing for facile conjugation to membranes and other solid supports. We have demonstrated that ultrafiltration membranes can be functionalized with aptamers for a variety of waterborne small-molecule toxins and contaminants and that this enables highly efficient capture of the target molecule upon sample filtration.[6] Compared to protein-based affinity reagents such as antibodies, aptamers have a number of characteristics that make them the ideal choice for this application.[7] In addition to their ease of bioconjugation, they can be reversibly denatured, they are stable at room temperature, and they can function in a wide range of buffers and biological matrices.[8]

Due to their facile chemical synthesis and modification, as well as their ability to undergo reversible thermal denaturation, aptamers have found wide use applications where their binding capabilities are used for therapeutics, targeting, or detection of specific analytes.[9-10] More recently, a small number of examples have also been reported where aptamers are attached to a solid support and used to bind and sequester their target molecule. Examples of these scaffolds include TiO_2 and PLA-PEG nanoparticles, Sepharose beads, and hydrogels.[11] While effective, most of these methods require expensive machinery for fabrication or implementation and they can be susceptible to corrosion or biofouling.

We have pioneered a new approach in which ultrafiltration membranes are functionalized with aptamers and we have demonstrated the ability of these membranes to bind and sequester

specific target molecules from a solution that is filtered through the membrane. Our initial work utilized this technology to remove waterborne toxins from environmental samples and drinking water, and in this chapter, we propose to explore the use of aptamer-functionalized membranes in a unique way so that we can gauge an idea of the capacity of application development of the membranes created. The simultaneous removal and degradation of bisphenol A by attaching Laccase enzyme to an aptamer-functionalized membrane. This way we can have a membrane that is regenerable on its own and it does not need further treatment to release the small molecule (often achieved by changes in pH or temperature).

With this application bearing our initial aptamer-functionalization membranes, we open up a window of research that can be applied in many fields, including materials, biological, environmental, and organic chemistry. Most importantly, SELEX has been developed for a wide range of small-molecules and proteins, providing a vast scope of important analytes for which our membrane different approaches could aid in achieving detection at biologically relevant concentrations.[12]

4.3 Self-Regeneration of Membranes Using Enzymes

The use of different biological tools to address the decontamination of water has been addressed in many different ways. Some researchers use inorganic or organic ways of removal technologies.[13-15] With the increase of interdisciplinarity, scientists have often paved ways of combining fields so that a problem can be solved in a rapid, systematic, and easy way.[16-17] With the current research that we have developed in the previous chapters, it is important to think in which way our current system can be used for a variety of applications.

In this case, we focus on the process after the removal of contaminants from water. We know that the small-molecule contaminants can be removed using aptamer-functionalized membranes. But there is still a drawback with this process, although it makes for a good removal strategy, the molecules removed remains intact,[18-19] for this reason it is important that we think about this

problem in a holistic way. Oftentimes, the membranes are regenerated by changes of pH or temperature (as we had shown in Chapter 2), but the problem is still there in the water with different pH or temperature. The aptamer, as it binds with small-molecules and toxins through non-covalent forces, the integrity of the small-molecule remains.[20] It is up to the person regenerating the membrane to dispose of this small-molecule contaminant in an appropriate form. This problem becomes even more persistent since we envision that the technology developed is of use in remote places and in a point-of-use way.[21] The consumer could make the small-molecule contaminants go back through the ecosystem because of careless disposal.

Taking into consideration the tools at our reach, we envision that we could tackle this problem by adding a degrading biomolecule to our system. Enzymes are adept for this purpose.[22] An enzyme is a protein that is able to act as a biological catalyst.[23] Specifically, laccase enzyme is able to serve our purpose because is able to degrade various organic contaminants.[24-25] Laccase is a copper-containing enzyme that consists of monomeric, dimeric and tetrameric glycoproteins.[26] Laccase is predominantly present in microorganisms, for example **Figure 4.1** shows the active laccase from *trametes versicolor*.[27] Laccase enzyme is mainly divided in terms

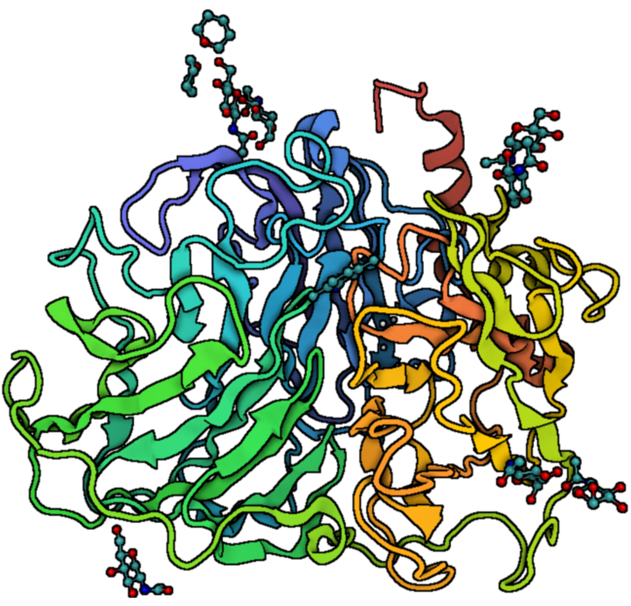


Figure 4.1: Active laccase from trametes versicolor. Laccase is a copper-containing extracellular enzyme that consists of monomeric, dimeric and tetrameric glycoproteins

of three categories based on their function: ring cleavage of organic compounds, degradation of biopolymers, and cross-linking structure of monomers.[28] Furthermore, this enzyme has been proven to work in both acidic and basic conditions. Laccase has the capability of degrade organic contaminants as it has been shown previously with examples like bisphenol A and Sulfamethoxazole.[29] The mechanism for degradation of these molecules can be found in **Figure 4.2**.[30] Although this has shown promise for the degradation of a big group of small-molecule organics, using enzymes in solution for water treatment has the same limitation as other techniques: the non-reusability, and high cost on single use.[31] Because of this various research had been switching gears towards the immobilization of this enzyme in different matrices, like for example yeast.[29] Furthermore, the efficacy of the degradation kinetics is dependent on the activity of the enzyme,[32] and if not, enough time is given to degrade the contaminant, low yields of depletion may cause this.

For this reason, and because of the development of our BPA depletion membrane using aptamer-functionalized membranes, we envisioned a system where we can achieve both, the depletion and degradation of contaminants by attaching membranes to the surface of the membrane. Due to the presence of NH_2 groups within the structure of the enzyme,[33] we thought that the attachment of the membrane could be achieved in the same manner as with the amine-modified aptamer. Due to the nature of the aptamers, first we can deplete the contaminant during

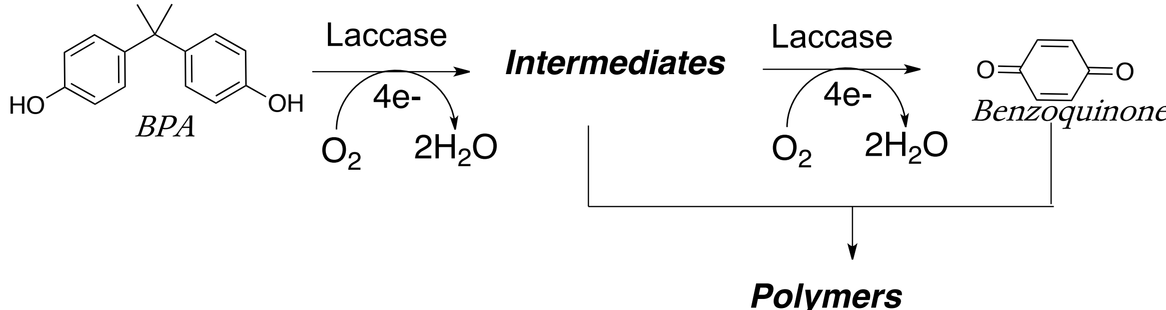


Figure 4.2: Degradation of BPA using laccase enzyme. The oxidative mechanism of the laccase degrades the molecule down to benzoquinone

the filtration process. This gives the enzyme enough time to act and degrade the contaminant of interest overnight.

There are various methods for laccase immobilization. These include adsorption, mesh embedding, micro-encapsuled embedding, covalent binding, self-immobilization, and two step combination.[34] All of these have various advantages and disadvantages, but there is a wide approval of covalent binding as this system sets forth various advantages including: a strong binding force between the laccase and the carrier, no laccase leakage or desorption, continuous use for a long time, and wide applications. Laccase is known to play significant role as a biocatalyst for micro-pollutants removal, but it can only do so after effective immobilization. The only drawback of current covalent binding immobilization techniques is that they are costly. We believe that the technology we have developed in this thesis, of using carboxylic acid moiety of poly-methacrylic acid, removes this gap and yields a process that improves the laccase stability, thermal, pH and storage operation.

The general method for the removal and degradation of bisphenol A can be found in **Figure 4.3**. The small molecule-aptamer and enzyme will both be attached at the same time to the

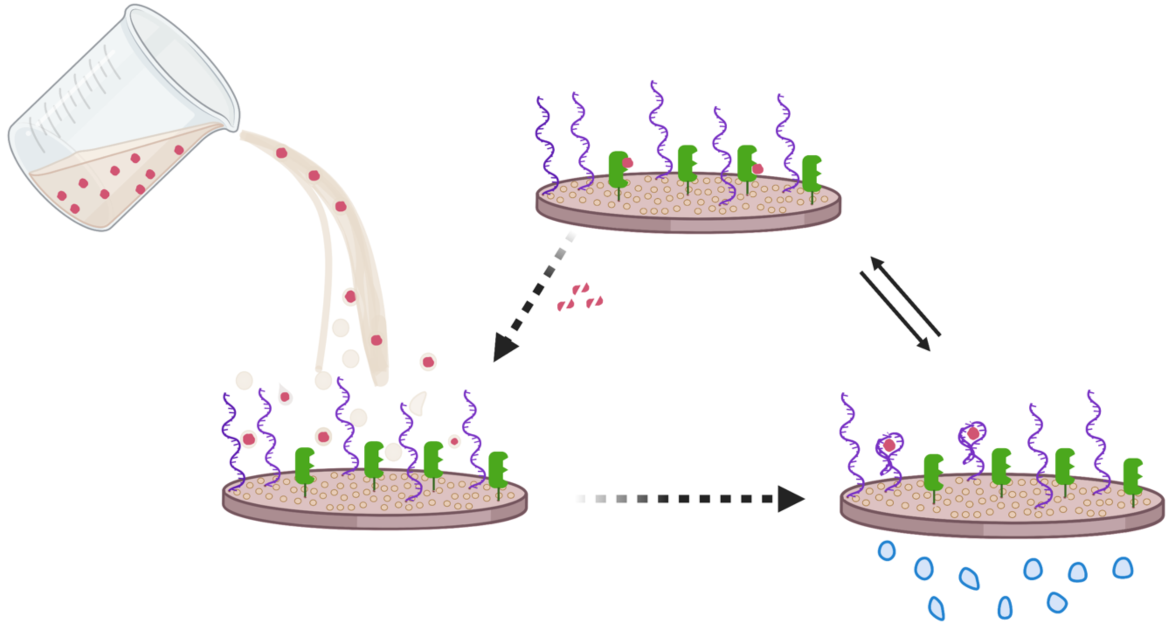


Figure 4.3: General procedure of activity of dual enzyme-aptamer-functionalized membranes. Initial depletion will happen due to the aptamer, while degradation will be set forth by the laccase enzyme.

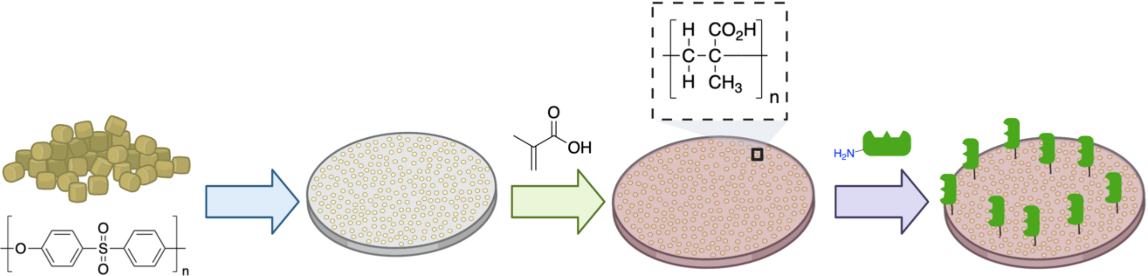


Figure 4.4: General reaction pathway for the generation of enzyme-functionalized membranes.

surface of the membrane. While initial depletion will be favored by the aptamer itself, the degradation will take part when these molecules reach equilibrium in solution overnight. This will allow the degradation of BPA to lower levels of small-molecule organics, making them not harmful anymore. We will test the reusability of this system by regenerating this membrane (leave in minimum solution of water) and repeating the depletion capacities. While we expect some kind of initial decrease in activity (just as seen in the experiments in Chapter 2), we expect to observe continued degradation an activity of this membrane for multiple rounds. This proof-of-concept will set forth a new are of investigation regarding the degradation of contaminants in water purification.

To start our investigation, we decided to focus on the attachment of the Laccase enzyme by out new method. For this reason, the membranes were prepared and synthesized as previously reported, and grafted as well. Then enzyme was attached on the ultrafiltration membrane by using

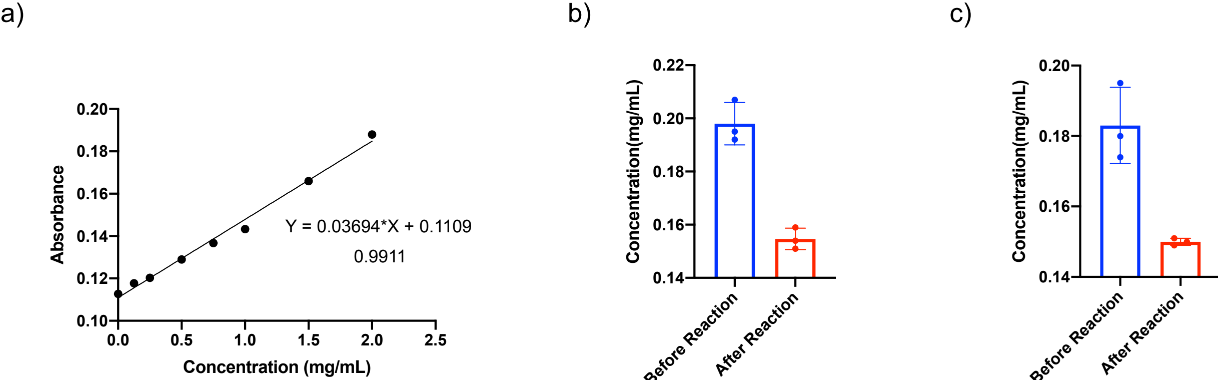


Figure 4.5: Initial experiments of attachment of enzyme (a) calibration curve to measure enzyme activity based on absorbance. (b) attachment efficiency of membrane with PEG 2000. (c) attachment efficiency of membrane with PEG 12000.

DMTMM as our coupling reagent and the coupling was done in MOPS Buffer. The reaction can be found in **Figure 4.4.** The membranes were done using Hydrophobic polymer PES and using PEG as an additive, in a similar fashion as reported previously. Then the ultrafiltration membrane was grafted using hydrophilic polymer poly-methacrylic acid. This yields carboxylic acid moieties that aid in the attachment with the amino groups of the enzyme. The attachment of the enzyme was monitored by absorbance of the solution containing the enzyme before and after reaction **Figure 4.5**.

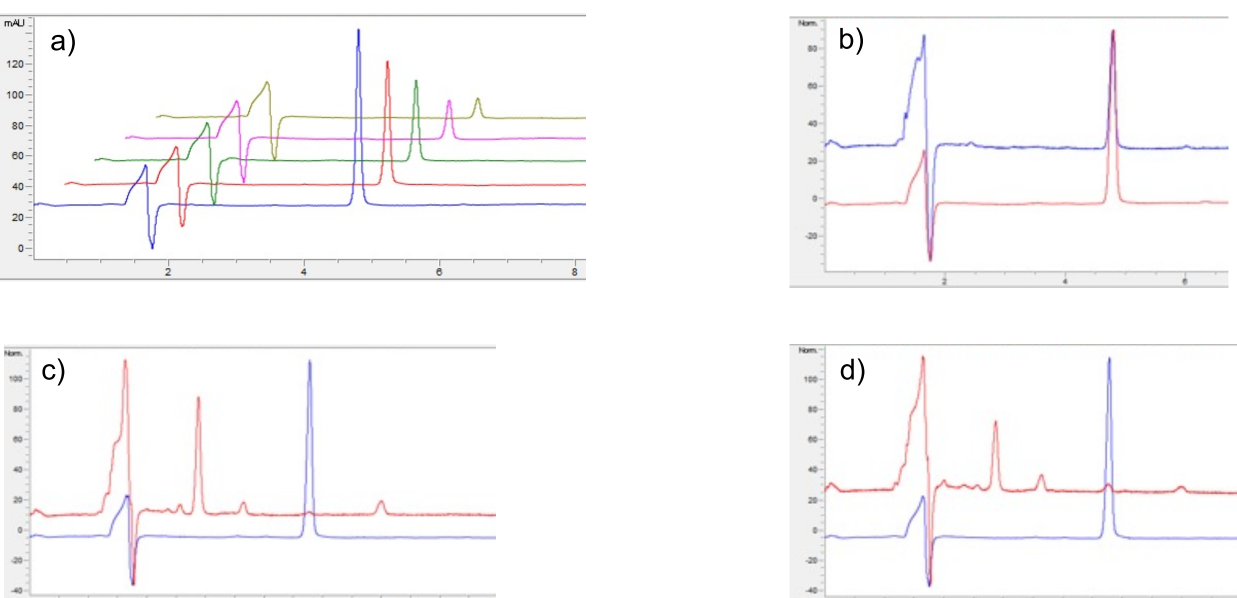


Figure 4.6: Degradation efficiency of enzyme-functionalized membrane towards the small-molecule contaminant BPA. (a) calibration curve of BPA. (b) control before and after with unfunctionalized membrane. (c) membrane MW 2000 depletion capacity (d) membrane MW 1200 depletion capacity. Blue before incubation, red after 24h incubation with each specified membrane.

We were able to check do these experiments in membranes MW 2000 and MW 12000 since they were proven to be the best yielding ones in terms of flux and bacteria removal. We were able to observe high yields when we used the methodology described: 3.52 mg of membrane for the membrane MW 2000 and 2.68 mg for the MW 12000. We wanted to make sure that the immobilization of the enzyme in the membrane did not affect the capability of the enzyme to degrade molecules of interest. For this reason, we decided to check for this by incubating the membranes that were made in BPA spiked water and observe it’s depletion efficiency over time. **Figure 4.6** contains the preliminary depletion efficiencies of this enzyme-functionalized

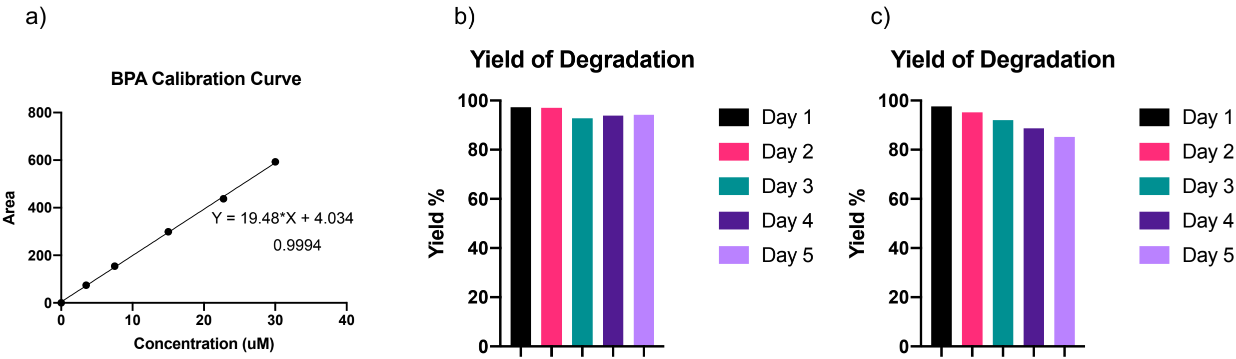


Figure 4.7: Reusability in the depletion capacity after 5 days. (a) BPA calibration curve (b) Degradation capacity by 2000 MW membrane (c) Degradation capacity by 12000 MW membrane.

membranes. We were able to observe that the removal of BPA was possible by not seeing any product at the same retention time after incubation in the enzyme-functionalized membranes, while the opposite effect was observed in the ungrafted membranes (BPA remained in solution). We also wanted to determine if this depletion can be continued for longer periods of time, as our final application will require the continued activity of these enzymes. This is because we believe that the activity of the enzymes will occur overnight, and it will be done during continuous days. So, we were able to repeat the BPA experiment for continuous days and we were able to observe that after 5 days the depletion capacity remains almost quantitative, which allows us to continue with our experimentation. These results can be found on **Figure 4.7**.

We were also curious to explore if the yield of attachment of enzyme would diminish if we used less enzyme. Taking in consideration the cost of our system, we want to make sure that we

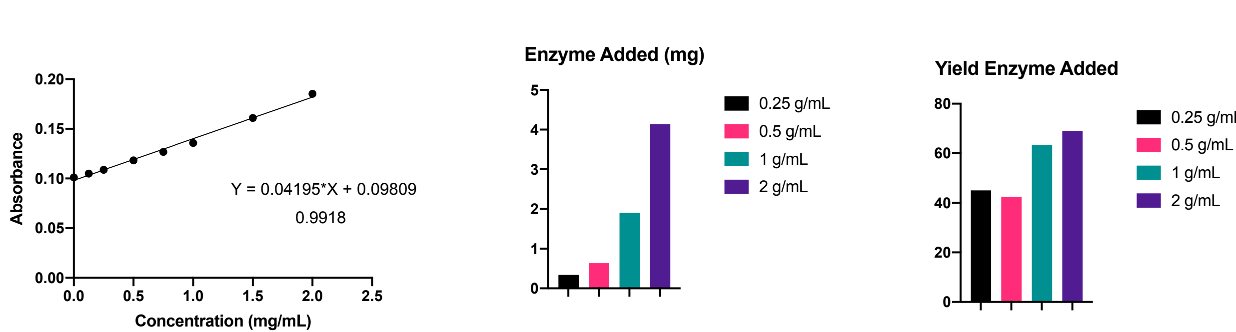


Figure 4.8: Enzyme attachment efficiency with varying concentrations of enzyme. (a) Laccase enzyme calibration, (b) enzyme added in mg (c) yield of each reaction.

optimize accordingly to use our resources to the maximum benefit. For this reason, we were able to check different concentrations of enzyme reaction and its effect on attachment yield. These results are summarized in **Figure 4.8**. We were able to observe that as you increase the

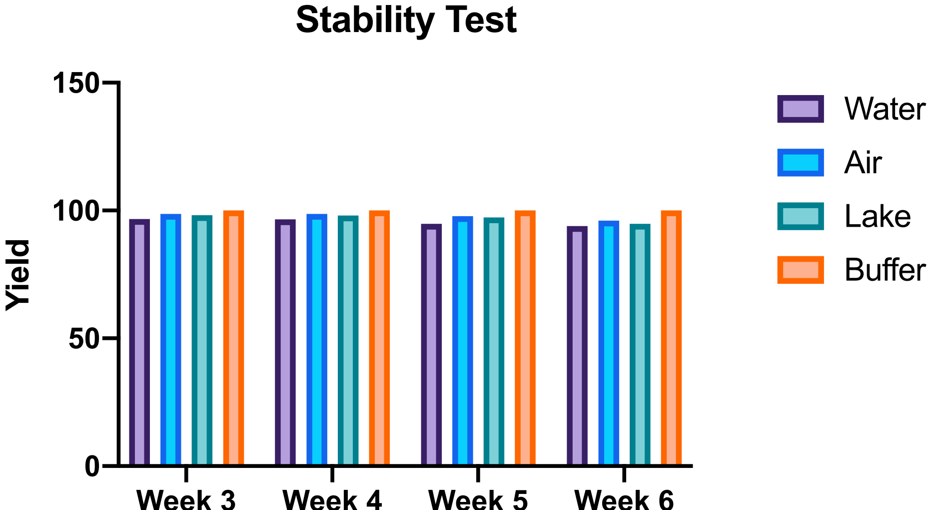


Figure 4.9: Stability test of enzyme-functionalized membranes after week in respective media.

concentration of enzyme in the reaction mixture the yield and the amount of enzyme and yield also increases. The highest yield was achieved by using concentration of 2 mg/mL so our subsequent studies will follow using that concentration.

We were also curious to observe how our enzyme-functionalized membrane behaves in different media of storage. We incubated an enzyme-functionalized membrane on air, lake water, miliQ and buffer and tested their ability to deplete BPA. We were able to do this for 6 weeks and we were able to observe a sustained depletion of BPA in all of the media. **Figure 4.9** denotes the degradation yield from week 3 onwards. These results confirm the versatility of using an enzyme mediated membrane for the depletion of small-molecule contaminants.

Once we had our enzyme system optimized, we were able to test if the attachment of both enzyme and aptamer were possible since they are both amine-modified and use the same coupling mechanism. For this to happen we followed the experimental method as outlined in **Figure 4.10**. In short, we start with 6mg of enzyme in 3mL solution and add fluorophore labeled

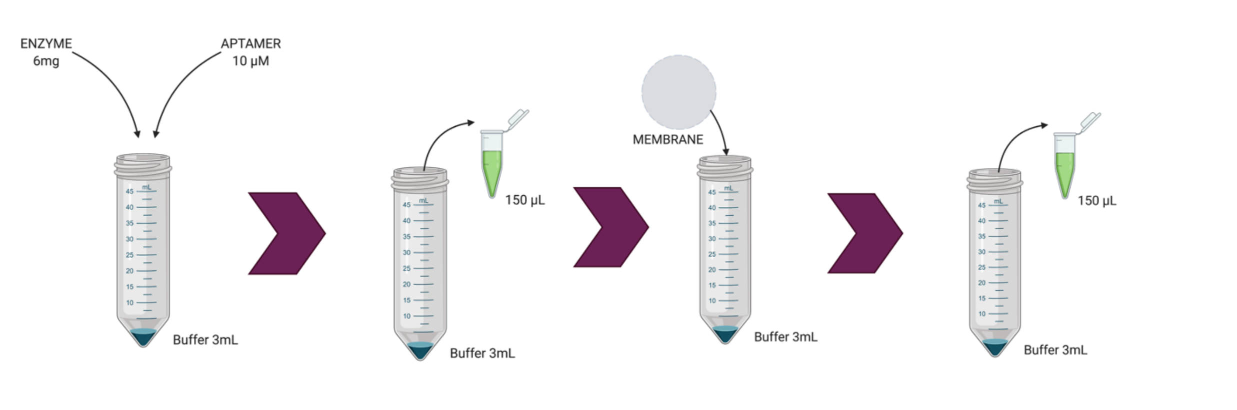


Figure 4.10: Attachment procedure for the simultaneous attachment of enzyme and aptamer.

amine-modified aptamer stock to make a final concentration of 10 µM. We take an initial aliquot to quantify the amount of enzyme and DNA attached to the membrane after reaction. We add the membrane which was activated with DMTMM solution (300 mM, 4mL) and shake in an orbital mixer for 24 hours. After this reaction time, the membrane is removed from solution and another aliquot is removed from the reaction mixture. These two aliquots are then read for both absorbance and fluorescence respectively (to measure the amount of aptamer and enzyme

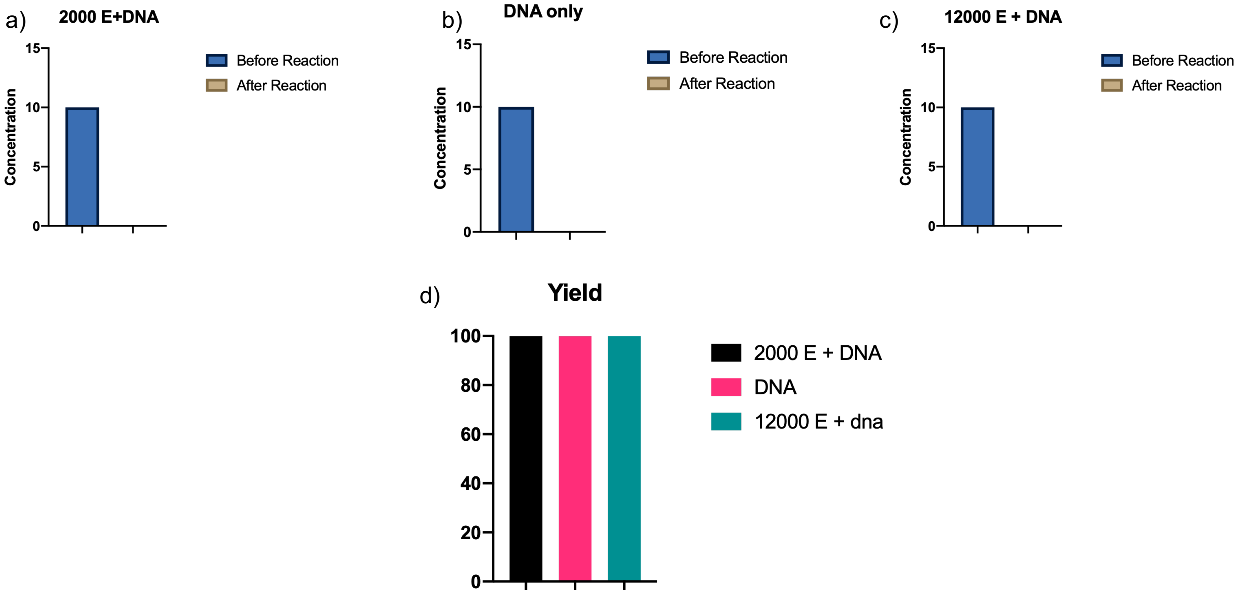


Figure 4.11: Attachment in terms of DNA to membrane (a) membrane 2000 MW (b) DNA only control (c) membrane 12000 MW (d) Yield and comparison of all aptamer attachment.

attached). We were able to observe that the attachment was favored by both the aptamer and enzyme. This is the first instance to the best of our knowledge where two different biomolecules are attached at the same time.

Figure 4.11 denotes the attachment to the membrane in terms of the aptamer. We are able to observe that a quantitative yield is observed for both membranes (2000 and 12000 MW) and they behave in the same manner than our control DNA only (**Figure 4.11b**). These results suggest that the attachment of the aptamer was possible.

In terms of enzyme attachment, we were able to observe that enough enzyme was attached in the same manner as our previous experiments. **Figure 4.12** shows the enzyme attachment yields. With this information we were able to observe that 3.22 mg of enzyme were attached to the 2000 MW membrane while 3.68 mg of enzyme were attached to the 12000 MW membrane.

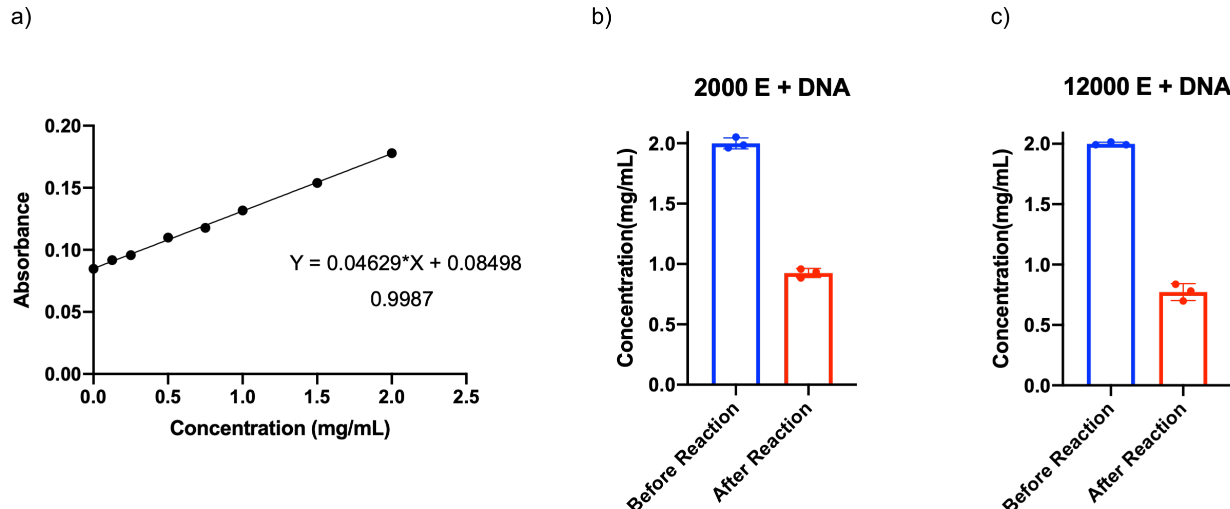


Figure 4.12: Yield of attachment in terms of enzyme. (a) Laccase enzyme calibration curve. (b) enzyme attachment for 2000 MW. (c) enzyme attachment for 12000 MW

Encouraged by the results obtained, we were able to check if the membrane obtained would be a good candidate for the depletion and degradation of small molecule contaminants. Taking into consideration that our proof of concept was to use BPA, we decided to first test the control of depletion with the aptamer-functionalized membrane alone. This is because we want to make sure that this is a synergistic type of approach where both entities (aptamers and enzymes) both

account for the depletion and degradation of the small-molecule contaminant. If the enzyme membrane is able to degrade on its own the necessity of the aptamers would not be necessary. **Figure 4.13** shows the depletion of BPA using an aptamer-functionalized membrane with 30 nmol of aptamer attached. Filtrations were done using a solution of 200 nM of tap water spiked with

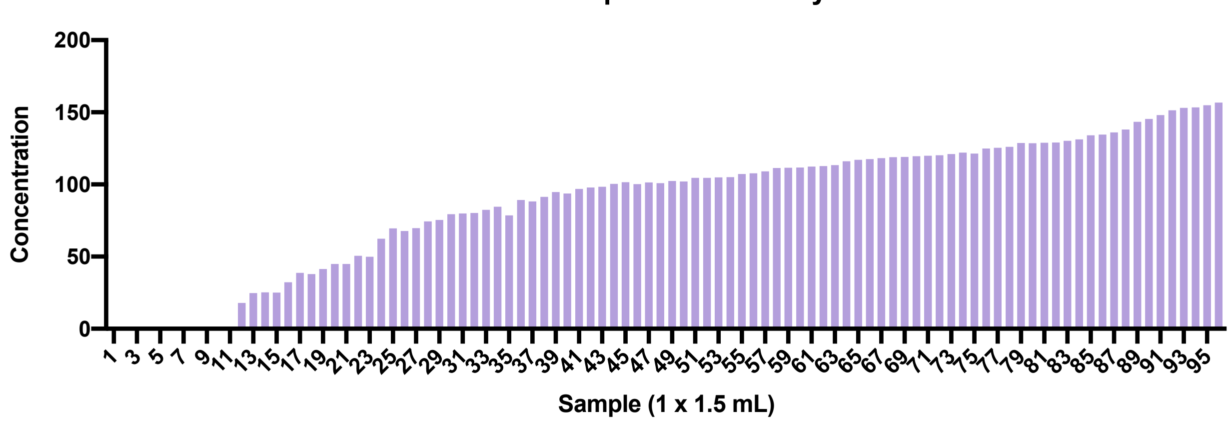


Figure 4.13: BPA depletion using an aptamer-functionalized membrane. It can be observed that the removal of this contaminant is due to the aptamer-small molecule sequestration.

BPA. Fractions were collected (1.5 mL each), which then were concentrated using a vacufuge and resuspended in 100 uL of water. This allowed for using HPLC to quantify the amount of BPA removed from solution by the aptamer functionalized membrane. It can be observed that the depletion happened faster in the first few fractions, and this is because there is more aptamer available in our membrane that hasn't been saturated with BPA.

Encouraged by the results obtained, we set forth of analyzing the enzyme only depletion of BPA. For this reason, we repeated the aforementioned experiment, but this time we used the enzyme-functionalized membrane. We expect that the removal of BPA would be not significant because the enzyme only has degradation capabilities and does not remove the molecule itself. We hypothesized that if we see a decrease of the amount of BPA in the solution it would be attributed to the degradation in situ as the filtration takes place. If we observe better depletion than with the aptamer-functionalized membrane, this will defeat the purpose of using these kinds of functional nucleic acids and we would be better off by optimizing the amount of attachment of

the enzyme to the membrane. The results of using an enzyme-functionalized membrane for the depletion of BPA is shown in **Figure 4.14**. It can be observed that the removal of small-molecule

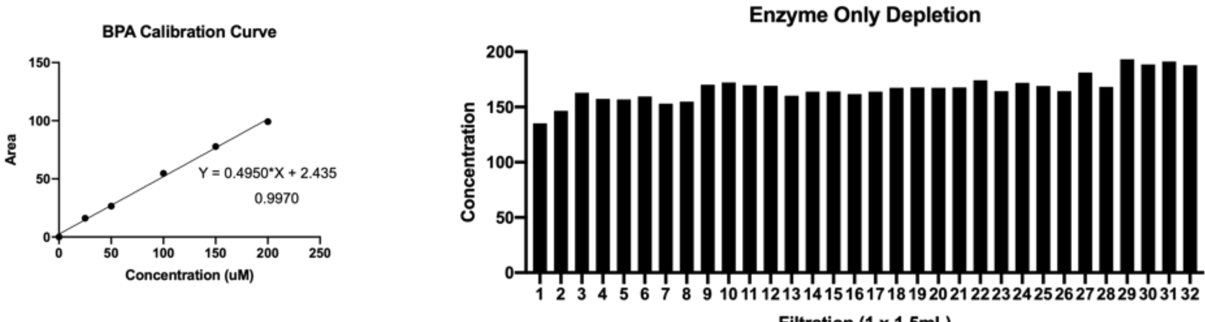


Figure 4.14: Depletion of BPA using an enzyme-functionalized membrane. (a) BPA calibration curve using HPLC. (b) BPA depletion for the first 32 fractions ~ 50 mL of contaminated water.

contaminant is minimal, only decreasing the concentration of the initial solution by 20% in the first few fractions until leveling it off back to the initial concentration on the later fractions. These results were very encouraging, because we then are going to be able to test out dual aptamer-enzyme functionalized membrane to (1) remove small molecules from water initially and (2) degrade molecules at the same time, in the same system, in friendly manner way.

4.4 <u>Conclusions</u>

The system developed here, sets forth for the first self-regenerable membrane that is able to no just remove small molecules of interest, but also degrade them. This research is able to attach both aptamers and membranes in a one-pot synthesis scenario and is able to take full advantages of the sequestration capabilities of aptamer as-well-as the degradation capabilities of membranes. This research is the first one that immobilized enzymes onto PES ultrafiltration membranes in a two-step synthesis (1) grafting and (2) functionalization. Furthermore, with the increase of the investigation of SELEX technologies and enzyme degradation capabilities, this system created can be used interchangeably so that specific decontamination scenarios can be met. This is especially crucial as discussed in previous chapters. The necessity of small-molecule contaminant depletion in remote areas is of much concern. The use of a point-of-use friendly

system is of upmost priority and this can only be done if the elements in the purification system can be durable and has long periods of shelf-life. We were able to achieve that by using immobilized enzymes (immobilization increases the shelf-life and activity) and aptamers (they have the innate capability of being regenerated). We believe that this technology will be of interest to the wider scientific community and the people that need water decontamination the most.

4.5 References

1. Farzin, L.; Shamsipur, M.; Sheibani, S., A review: Aptamer-based analytical strategies using the nanomaterials for environmental and human monitoring of toxic heavy metals. *Talanta* **2017,** *174*, 619-627.

2. Ding, F.; Gao, Y.; He, X., Recent progresses in biomedical applications of aptamer-functionalized systems. *Bioorganic & Medicinal Chemistry Letters* **2017,** *27* (18), 4256-4269.

3. Kaur, H., Recent developments in cell-SELEX technology for aptamer selection. *Biochimica et Biophysica Acta (BBA) - General Subjects* **2018,** *1862* (10), 2323-2329.

4. Zhuo, Z.; Yu, Y.; Wang, M.; Li, J.; Zhang, Z.; Liu, J.; Wu, X.; Lu, A.; Zhang, G.; Zhang, B., Recent Advances in SELEX Technology and Aptamer Applications in Biomedicine. *International Journal of Molecular Sciences* **2017,** *18* (10).

5. Qiao, L.; Wang, H.; He, J.; Yang, S.; Chen, A., Truncated affinity-improved aptamers for 17β-estradiol determination by AuNPs-based colorimetric aptasensor. *Food Chemistry* **2021,** *340*, 128181.

6. Romero-Reyes, M. A.; Heemstra, J. M., Small-Molecule Sequestration Using Aptamer-Functionalized Membranes. *ACS Materials Letters* **2019,** *1* (5), 568-572.

7. Arshavsky-Graham, S.; Urmann, K.; Salama, R.; Massad-Ivanir, N.; Walter, J.-G.; Scheper, T.; Segal, E., Aptamers vs. antibodies as capture probes in optical porous silicon biosensors. *Analyst* **2020,** *145* (14), 4991-5003.

8. Belleperche, M.; DeRosa, M. C., pH-Control in Aptamer-Based Diagnostics, Therapeutics, and Analytical Applications. *Pharmaceuticals* **2018,** *11* (3).

9. Keefe, A. D.; Pai, S.; Ellington, A., Aptamers as therapeutics. *Nature Reviews Drug Discovery* **2010,** *9* (7), 537-550.

10. Zhou, J.; Rossi, J., Aptamers as targeted therapeutics: current potential and challenges. *Nature Reviews Drug Discovery* **2017,** *16* (3), 181-202.

11. Bilibana, M. P.; Citartan, M.; Yeoh, T. S.; Rozhdestvensky, T. S.; Tang, T.-H., Aptamers as the Agent in Decontamination Assays (Apta-Decontamination Assays): From the Environment to the Potential Application In Vivo. *Journal of nucleic acids* **2017,** *2017*, 3712070-3712070.

12. Komarova, N.; Kuznetsov, A., Inside the Black Box: What Makes SELEX Better? *Molecules* **2019,** *24* (19), 3598.

13. Deborde, M.; von Gunten, U., Reactions of chlorine with inorganic and organic compounds during water treatment—Kinetics and mechanisms: A critical review. *Water Research* **2008,** *42* (1), 13-51.

14. Marais, S. S.; Ncube, E. J.; Msagati, T. A. M.; Mamba, B. B.; Nkambule, T. T. I., Comparison of natural organic matter removal by ultrafiltration, granular activated carbon filtration and full scale conventional water treatment. *Journal of Environmental Chemical Engineering* **2018,** *6* (5), 6282-6289.

15. Hua, G.; Kim, J.; Reckhow, D. A., Disinfection byproduct formation from lignin precursors. *Water Research* **2014,** *63*, 285-295.

16. Nasrollahzadeh, M.; Sajjadi, M.; Iravani, S.; Varma, R. S., Green-synthesized nanocatalysts and nanomaterials for water treatment: Current challenges and future perspectives. *Journal of Hazardous Materials* **2021,** *401*, 123401.

17. Zhu, X.; Jassby, D., Electroactive Membranes for Water Treatment: Enhanced Treatment Functionalities, Energy Considerations, and Future Challenges. *Accounts of Chemical Research* **2019,** *52* (5), 1177-1186.

18. Zheng, J.; Wang, Z.; Ma, J.; Xu, S.; Wu, Z., Development of an Electrochemical Ceramic Membrane Filtration System for Efficient Contaminant Removal from Waters. *Environmental Science & Technology* **2018,** *52* (7), 4117-4126.

19. Shekhar, M.; Shriwastav, A.; Bose, P.; Hameed, S., Microfiltration of algae: Impact of algal species, backwashing mode and duration of filtration cycle. *Algal Research* **2017,** *23*, 104-112.

20. Chen, J.; Li, N.; Wang, X.; Chen, J.; Zhang, J. Z. H.; Zhu, T., Molecular mechanism related to the binding of fluorophores to Mango-II revealed by multiple-replica molecular dynamics simulations. *Physical Chemistry Chemical Physics* **2021,** *23* (17), 10636-10649.

21. Gunda, T.; Hess, D.; Hornberger, G. M.; Worland, S., Water security in practice: The quantity-quality-society nexus. *Water Security* **2019,** *6*, 100022.

22. Yang, S.; Hai, F. I.; Nghiem, L. D.; Price, W. E.; Roddick, F.; Moreira, M. T.; Magram, S. F., Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: A critical review. *Bioresource Technology* **2013,** *141*, 97-108.

23. Alves, N. J.; Moore, M.; Johnson, B. J.; Dean, S. N.; Turner, K. B.; Medintz, I. L.; Walper, S. A., Environmental Decontamination of a Chemical Warfare Simulant Utilizing a Membrane Vesicle-Encapsulated Phosphotriesterase. *ACS Applied Materials & Interfaces* **2018,** *10* (18), 15712-15719.

24. Ba, S.; Arsenault, A.; Hassani, T.; Jones, J. P.; Cabana, H., Laccase immobilization and insolubilization: from fundamentals to applications for the elimination of emerging contaminants in wastewater treatment. *Critical Reviews in Biotechnology* **2013,** *33* (4), 404-418.

25. Madhavi, V.; Lele, S. S., LACCASE: PROPERTIES AND APPLICATIONS. *BioResources; Vol 4, No 4 (2009)* **2009**.

26. Gasser, C. A.; Ammann, E. M.; Shahgaldian, P.; Corvini, P. F. X., Laccases to take on the challenge of emerging organic contaminants in wastewater. *Applied Microbiology and Biotechnology* **2014,** *98* (24), 9931-9952.

27. Majcherczyk, A.; Johannes, C.; Hüttermann, A., Oxidation of Polycyclic Aromatic Hydrocarbons (PAH) by Laccase of Trametes Versicolor. *Enzyme and Microbial Technology* **1998,** *22* (5), 335-341.

28. Xu, P.; Du, H.; Peng, X.; Tang, Y.; Zhou, Y.; Chen, X.; Fei, J.; Meng, Y.; Yuan, L., Degradation of several polycyclic aromatic hydrocarbons by laccase in reverse micelle system. *Science of The Total Environment* **2020,** *708*, 134970.

29. Chen, Y.; Stemple, B.; Kumar, M.; Wei, N., Cell Surface Display Fungal Laccase as a Renewable Biocatalyst for Degradation of Persistent Micropollutants Bisphenol A and Sulfamethoxazole. *Environmental Science & Technology* **2016,** *50* (16), 8799-8808.

30. Daâssi, D.; Prieto, A.; Zouari-Mechichi, H.; Martínez, M. J.; Nasri, M.; Mechichi, T., Degradation of bisphenol A by different fungal laccases and identification of its degradation products. *International Biodeterioration & Biodegradation* **2016,** *110*, 181-188.

31. Ba, S.; Vinoth Kumar, V., Recent developments in the use of tyrosinase and laccase in environmental applications. *Critical Reviews in Biotechnology* **2017,** *37* (7), 819-832.

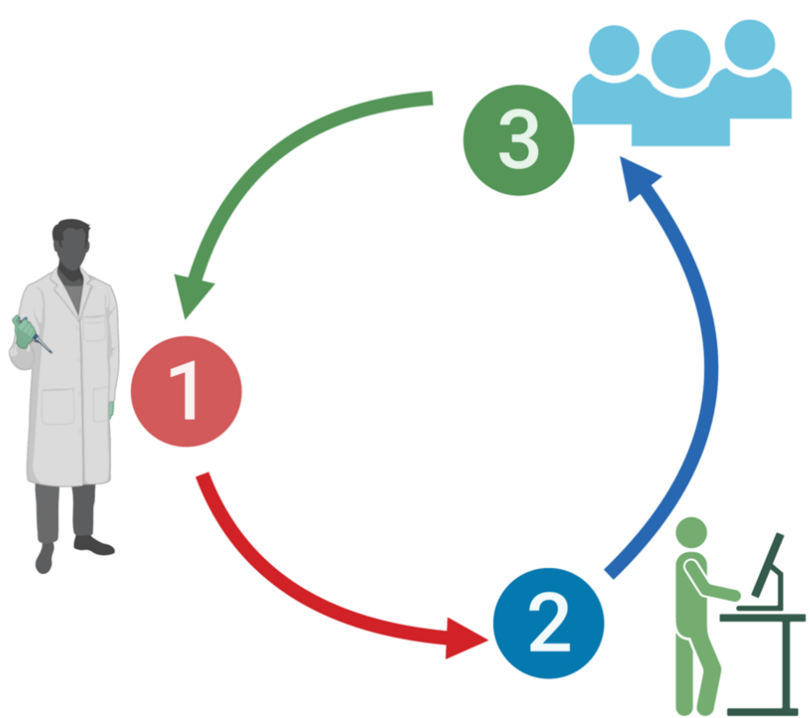
32. Lassouane, F.; Aït-Amar, H.; Amrani, S.; Rodriguez-Couto, S., A promising laccase immobilization approach for Bisphenol A removal from aqueous solutions. *Bioresource Technology* **2019,** *271*, 360-367.

33. Zhang, Y.; Hu, P.; Muhammad, Y.; Tang, Y.; Shao, S.; Gao, Z.; Wang, J.; Wang, R.; Hu, Y.; Kuang, L.; Zhao, Z.; Zhao, Z., High-density immobilization of laccase on hollow nano-sphere NH2-MIL88(Fe) host with interfacial defects to improve enzyme activity and stability for remazol brilliant blue R decolorization. *Chemical Engineering Journal* **2021,** *405*, 127003.

34. Zhou, W.; Zhang, W.; Cai, Y., Laccase immobilization for water purification: A comprehensive review. *Chemical Engineering Journal* **2021,** *403*, 126272.

Chapter 5

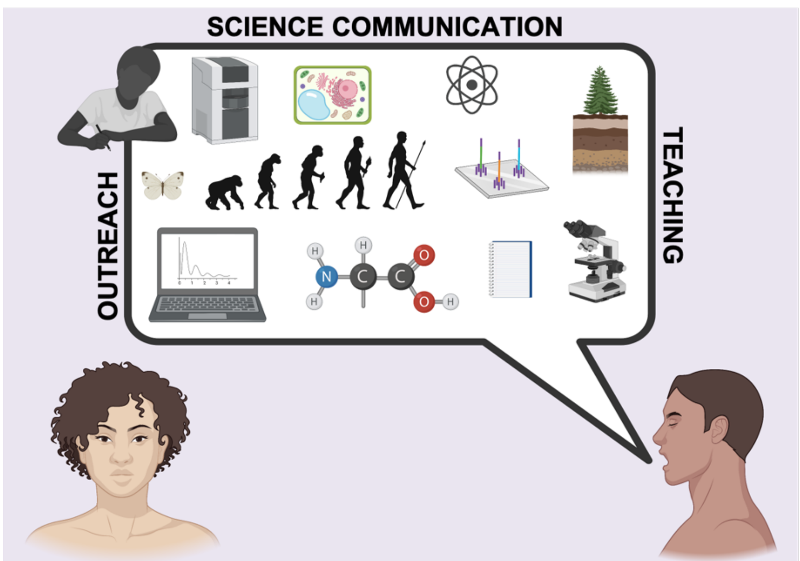
Graduate School is More Than Research: A Path of Professional Development.



In this chapter, we reflect on the professional development activities that go beyond doing outstanding research: science communication, teaching and outreach.

5.1 Abstract

Graduate School is most commonly defined as a program to get specialized advanced degrees that build upon what was learned in undergraduate education. In the natural sciences, this often translates into gaining specialized research experience and getting publications in peer reviewed journals. But is graduate school just that? Although it is a great way to get scientific knowledge, graduate school can also serve as a training platform to reach your future academic goals. Students must overcome challenges that they may be facing, and graduate school may offer the training to get a holistic experience that can help in all areas of development. In this chapter I provide a detailed description of my own working system to accomplish the preparation needed to achieve a great publication record, work-life balance, and training to attain success. The things discussed in this chapter come from firsthand experiences that I have had in my graduate career and my example is not the only formula for success. There are various paths that can be taken, and the objective is to find what you are passionate about and use graduate school as a preparation to achieve that goal. This chapter is divided in three primary sections: (1) science communication, (2) teaching, and (3) outreach. I explain how the combination of these three different experiences allowed me to have holistic training that allowed me to pursue my dream of becoming mentor, teacher, and researcher.



5.2 Introduction

I have envisioned my dissertation journey as a critical juncture where I not only gain essential research skills and investigate a scientific problem, but also as a path to give to those I mentor and teach. My doctoral research focuses on developing molecular sequestration techniques by harnessing the selective properties of nucleic acids and developing a functionalized polymeric membrane platform to enable the targeted capture and removal of toxins. Working on this project allowed me to develop a range of skills in organic, biological, and materials chemistry which equipped me to serve as a mentor to several undergraduate and graduate students in the laboratory courses I taught and within my own lab. Teaching these courses, in conjunction with a set of teaching workshops, has allowed me to master the use of radical candor, which is the key to providing effective feedback to students.

The training that I received prepared me to develop and teach an entirely new class, which was so successful among students that it has been permanently established in the chemistry curriculum at Emory University. This class, which I taught as the sole instructor of record with support from a Dean's Teaching Fellowship, emphasizes students' critical thinking by utilizing relevant primary research literature to help them understand the chemical changes that are currently shaping our environment. My training and development, then, leave me well prepared to teach both general and advanced levels of chemistry, with an emphasis on introductory and general, organic, biological, and environmental chemistries.

Aside from lecturing and research, I am devoted to advancing scientific knowledge to everyone, including the general public and underrepresented groups. I have pursued three primary goals: increase awareness and accessibility of science to the general public, provide international students a sense of belonging, and increase the number of underrepresented minorities in college. To achieve these goals, I participated in many outreach events that allowed me to enhance my science communication skills. For example, I was able to generate enthusiasm

and overall awareness of science by teaching elementary school kids about my research by developing an interactive game that taught them complex topics like aptamers. International students culturally and academically enrich our society, yet they face many hurdles (e.g. acceptance by peers, language barriers) that prevent them from experiencing belonging. To improve their contributions and engagements, I established a chartered support group for international students. This successful group works to dismantle barriers and provides a space where students can come together to voice their concerns and teach us about their culture. A powerful technique to further the advancement of underrepresented groups in science requires that current students and mentors actively meet with future students to discuss our passion, to facilitate encouragement, and work to address their concerns. This prompted me to serve as an EDGE (Emory Diversifying Graduate Education) Ambassador for the past three years, informing high school students about college and college students about graduate school. Participating in my outreach and DEI activities has taught me how to communicate with patience and kindness to my fellow colleagues, students, and the public.

It is not simply the combination of different approaches that has allowed me to have a holistic graduate experience and the successes that I have attained in the last few years. It all starts with a plan and a commitment to keep pushing though roadblocks that you might encounter, and of course, never giving up. In the next few sections, I will disseminate specifics on these different elements and how they worked in combination to prepare me for my long-term career goals.

5.3 Science Communication

Science communication is an art. Trying to explain a variety of complex scientific concepts to an audience with the goal of informing, educating, and raising awareness, is not an easy task. I did not know about this art until I arrived in the US and wanted to practice the way in which I present my science.

With the goal in mind of improving my English, I decided to participate and get involved in any conference, poster presentation, or workshop that I could. This allowed me to first move past the nervousness phase of presenting in front of an audience (which can be dauting to everyone) and second, to better prepare for the next presentation. There is a beauty in the way people ask questions (no matter the scientific level) that allows you to answer that question next time you present. This allowed me to be more confident at presenting my science and always keep improving the way I talk about it.

It wasn't until my third year in graduate school that I realized what science communication could do. First, I was able to attend my first science communication conference: COMSCICON Atlanta 2019. This conference was quite different from the usual conferences and workshops I had been attending thus far, as this conference mainly focused on raising the awareness of science topics amongst the general public. At the end of the conference, I was able to explain my science to the general public in a concise and effective manner. This allowed me to go outside of my comfort zone and really dig deeper into understanding my scientific topic and being able to explain it in such a manner that anyone could understand.

My first opportunity to use my science communication skills publicly came shortly after that, when I competed and won the Wunderbar Science Slam. A science slam is a competition where you have to creatively explain your research to a general audience (consisting of adults or kids) in under 10 minutes. I decided to accomplish this with a game that even kids could understand. This game was based on the cookie monster, and participants had to defeat the

cookie monster by trapping him with a net, which related to using aptamers to capture a specific small-molecule target. After the game, I realized how powerful it was to have elementary school kids understand what an aptamer was and how it contributes to water purification.

A few months before in the summer of 2019, I was able to give my first ever seminar talk at a research conference. This conference, The Nucleosides, Nucleotides and Oligonucleotides GRS brings together graduate students and postdocs, as a chance for them to highlight their work in an informal setting before a weeklong conference (GRC) with professors and established researchers. I was one of the 14 speakers that were able to present their research for 15 minutes. Something interesting that happened just right before everyone started presenting: they said that they were going to select the best presentation and invite that individual to present again during the actual conference on the first night after the keynote speaker. In front of over 200 people! This made me even more nervous. I was happy just relaying my first presentation. No matter what, I told myself, I will give it my best. I was able to present with no complications; I stayed calm and was able to effortlessly answer all the questions. I was happy that I was done. Little did I know that the next day my life would change forever. I thought I was safe, meaning there were other presentations far better than mine. It was time for the live vote, and I was feeling like a nervous wreck; this voting process lasted a good five minutes and it turns out I won with only a 6-vote difference, making me feel even more nervous. I had to present before 200+ people, and all of them were professors and industry people. It was definitely an intimidating experience, even though I presented the same information multiple times before. My nerves dissipated as I got into the talk, and by the end I was able to give a successful conference talk twice!

These two instances that I presented might portray me as a great communicator, even so this was not always the case. I always love, love, love talking, but these recent public speaking events and success come after countless practices and less-than-perfect attempts. I owe a great portion of this achievement to Emory because they provided me many opportunities to develop my presentation skills and I jumped at every opportunity to do so. Opportunities that Emory

provides includes conferences, Atlanta science festival events, recruitment weekends…all kinds of things. During my time at Emory, I have been able to give over 13 poster presentations to different audiences and that is what allows me to be able to explain my science at the level I do now, with still significant room for improvement. I can always grow, but I could not be able to get this far without the opportunities I've had during my PhD.

5.4 Teaching

My teaching is motivated by the core principle that learning has the power to inform, and thus bring about change. Education can propel a society to move forward, and the methods and techniques for teaching play a vital role in this progress. I developed these beliefs throughout my own education and as a TA and instructor for a variety of courses. In adapting my own teaching and materials to support a diverse audience with different learning styles, I promote active learning, which can include the appropriate use of technology, problem-based learning, flipped classroom organization, and small-group discussions. Using an integrated active learning methodology combined with creative teaching pedagogies will greatly promote everyone's understanding of fundamental theories in chemistry, regardless of their previous background expertise or their future career interests. Adapting this philosophy has allowed me to teach a variety of courses including introductory and advanced organic chemistry, biochemistry, general chemistry, and environmental chemistry.

I have embraced an integrated teaching style that pulls from a variety of approaches with the aim of engaging students. For example, I know there is merit in traditional lecture-based instruction, but it should be combined with newer technology-based learning platforms to better support the entering generation of students. I have implemented innovative tools such as Explain EDU, which is beneficial for more mathematical chemistry problems because you can easily show how to work through a problem. Working through such problems and other discussions benefits

the student because they can immediately apply what they have learned. These types of activities allow the students to feel more comfortable discussing their questions and concerns. In another biochemistry class that I helped develop and teach, students were simultaneously able to understand current biochemical research and basic chemical concepts related to biochemistry through a flipped classroom approach, which allows the student to take control of the classroom and learn concepts by teaching them to the class. I feel confident that this method helped my students achieve the course goals because of their overall success on standard examinations.

Furthermore, I have completed special training to develop effective online courses and explore digital tools to develop pedagogical methods for research and training both in and outside the classroom. From this, I incorporated a combination of synchronous and asynchronous activities in my classes to maximize student performance without letting them feel overwhelmed. While I have ensured that I can explain concepts in a simple manner by following a non-traditional teaching method, I aimed to ensure that my teaching arsenal also contained adaptable teaching pedagogies. For this reason, I have also implemented a problem-based learning approach, which includes using real-world problems to explain core concepts. I followed the problem-based learning scenarios with small, easily digestible lectures on new concepts by making connections to the concepts that students learned in their pre-requisite courses.

Although I was able to gain some training in both TAing and learning about using online tools and techniques in my teaching, my main source of learning was when I was awarded the prestigious Dean’s Teaching Fellowship at Emory University. With this fellowship, students in their final years of graduate school are able to teach a course as the instructor of record. The beauty of this opportunity is that you teach a course like people in the professional world do: having full control of the classroom, activities, and assignments. Usually, this kind of fellowship translates to teaching an introductory level in chemistry that has an established cohort of faculty that guide you through your first instructor experience. In my case, and after I got mentally prepared to teach an introductory class, the chemistry department really liked the syllabus that I proposed for the upper-

level elective: Chemistry and the Environment and they asked me if I could teach that one. I really enjoyed making that syllabus, so I was delighted to teach that class because it paired the concepts of chemistry and the environment, both of which I am passionate about. The syllabus was developed for in-person classes, but right before starting the semester, the school decided to switch the majority of its classes to a remote format due to the COVID-19 pandemic. I had to quickly re-format the syllabus to include synchronous and asynchronous activities to make sure that I maximized student's performance while not burning them out.

With all the technologies and methods outlined above, I have seen an increase in student engagement, most particularly in my advanced chemistry class, **Chemistry and the Environment**, that I instructed in Fall 2020. During this class, the students appreciated and clearly understood the chemical changes that are facing our environment by distilling the information from primary literature sources and focusing on a current environmental issue, such as carbon dioxide capturing and decontamination of textile wastewater. This approach promoted knowledge proficiency and application because it helped the students develop critical thinking and problem solving by actively applying their knowledge of what they were learning, rather than straight memorization. With these skills, students can be more judicious and apply their knowledge more easily in practical, challenging situations. Throughout my career I have obtained teaching experiences in introductory and advanced courses, which has helped me to understand the differences between the depth (specificity) and/or generality of the material that I should cover depending on the course objectives.

As a teacher and mentor, I want to ensure that I make my students comfortable and facilitate interest in the subject that I am teaching and demonstrate how the knowledge can be utilized in whichever career path they are willing to pursue. I am able to do this by implementing different metrics in my classes allowing students to voice their opportunities and concerns. Through a mid-semester survey for one of my classes, I was able to observe that the students were really appreciative of the writing assignment that required them to concisely summarize a

research paper. The students recognized that this was an important skill that can be transferred and applied in every branch of their careers. In STEM fields, we often find an underrepresentation of minority groups and we must actively seek to change this dynamic. I have various strategies in place to make sure that I can do this effectively. This includes (i) providing a space (e.g. surveys, office hours, emails) to ensure they can voice their concerns and questions about STEM fields (ii) establishing mentorship and networks with these students to allow them to converse with their mentors at any time regarding their concerns and questions. I was able to recently do this in a class setting where I had many diverse students, with a majority from an underrepresented background. Every student had a different career interest, and a few students also had some challenges to understanding the material. I made myself available to everyone and had metrics in place to gauge whether the students were interested and engaged. I was able to mentor most students who sought me out for advice regarding STEM careers and their concerns, along with requests for more information regarding scholarships and additional STEM resources.

Due to the success of the class: from implementing new pedagogies and tools, to using technology in (despite moving into an online format due to COVID-19), I was awarded the Dobes Outstanding Teaching Award. This award will allow me to deliver a course to graduate students about how to present their research by incorporating unique creative and persuasive elements. My unique training and teaching experiences have taught me different pedagogical tools, learning styles, and engagement tools, all of which serve to enhance and promote a creative learning environment.

5.5 Outreach

Diversity is a word that has multiple connotations. A person cannot be "diverse" *per se*, but rather diversity relates to the unique characteristics of individuals in groups or communities. Oftentimes, people, including myself, struggle with what diversity entails. As a Hispanic, brown,

person of Spanish speaking origins, I often have found myself wondering who I really am within my community. To overcome this confusion that other groups of people may face, I sought to demystify this term and help others find a place of belonging (finding a support group for international students, participating in Emory's EDGE, and science communication). My efforts towards outreach can be divided into three distinct categories:

Increasing Access of Science to the General Public

Increasing access to knowledge and impact of science among the public is important, and this can be achieved through improving overall *science communication*. I have worked on mastering my science communication through conferences, workshops, and fellowships. I am committed to demystifying basic science, chemistry, and my specific research to diverse groups of people. For example, in order to broaden the impact in my university, I partnered with the Spanish and Portuguese Department at Emory University and worked to create science activities based on general principles and my research, trivia, and games for students learning Spanish. Through additional workshops, guest speakers, and Spanish conversation hours, I also held scientific discussions and debates with the general population (non-science major students). I was able to measure a positive impact because I was able to recruit a few students into the Chemistry program (major, minor, and undergraduate research) after they were intrigued from these activities.

International Graduate Student and Scholars (IGSS)

Establishing a network and a support group as a student and/or researcher is very important. I discovered that this is increasingly difficult for international students, due to the multiple aspects, such as language barriers, new country, isolation from family/friends…etc. that are involved for them to adapt to a new country. I felt that Emory needed a group that connects and brings everyone, especially international students together. Keeping this mission in mind, I spearheaded

and established a funded organization: *International Graduate Student and Scholars (IGSS)*. Ever since its inception in 2018, IGSS has made it its mission to continuously strive to break barriers between cultures and foster intercultural bonding between international students and domestic students, through different activities, workshops, and events. We were soon to be able to mobilize and garner participation from other departments, and now, we have officially been recognized as the main international student group at Laney Graduate School and the point-of-contact graduate organization by the International Student and Scholar Services of Emory University.

Emory Diversifying Graduate Education (EDGE)

Increasing opportunities for those that are interested in pursuing undergraduate and graduate education is essential. This is especially important for people that are from marginalized groups because they face challenges (due to a variety of factors) in continuing their education. In order to bring visibility to science education, I decided to become an ambassador for Emory Diversifying Graduate Education (EDGE). EDGE aims to aid Emory University by increasing exposure, admissions, enrollment, successful matriculation, and graduation of diverse scholars. Through this program, we were able to recruit diverse students to Emory by participating in fairs, conferences, etc. and talking to prospective students about undergraduate and graduate school. It was to my surprise that students do not know the array of opportunities that open up with a graduate degree and are often concerned about the costs that this degree may potentially require. I focused on closing such gaps by attending these activities and mentored a couple students by helping them navigate their concerns and addressing them with multiple resources (e.g. scholarship information, TA information, external fellowships in their field)

All of these outreach experiences have also helped in my teaching. Every person is different, especially when it comes to learning. Each student has a unique method for learning, and as an instructor (and mentor) we must strive to meet every student's needs to help them grow and reach their goals. I have implemented this philosophy by using multiple engagement

strategies in each of my lectures, class activities and assignments. It is also important for students to envision themselves in their professors and I want to be a role model for students of underrepresented backgrounds.

5.6 Conclusion

The combination of my science specialty, with science communication, teaching, and outreach, allowed me to gain a unique set of experiences that in turn allowed me to improve my own professional development and bridge gaps in ways that are not normally seen in academia. I was fortunate to be able to develop as a science communicator and teacher during graduate school and this allowed me to achieve my goal of becoming a professor. There are just a few teachers that I have encountered in my education that are great science communicators. Unfortunately, not all educators value this skill, and this often leads to low retention of students (especially minorities) in the sciences. I will strive to make sure that all my students understand the topics at hand and use science communication to relate what they are learning to an everyday experience.

Science communication is in a way teaching, and to be an effective educator you need to be an effective science communicator. Outreach allowed me to see that everyone is different and therefore as a future professor, I should work with my students in an individual way that maximizes their performance by focusing on their unique needs, culture, and learning styles.

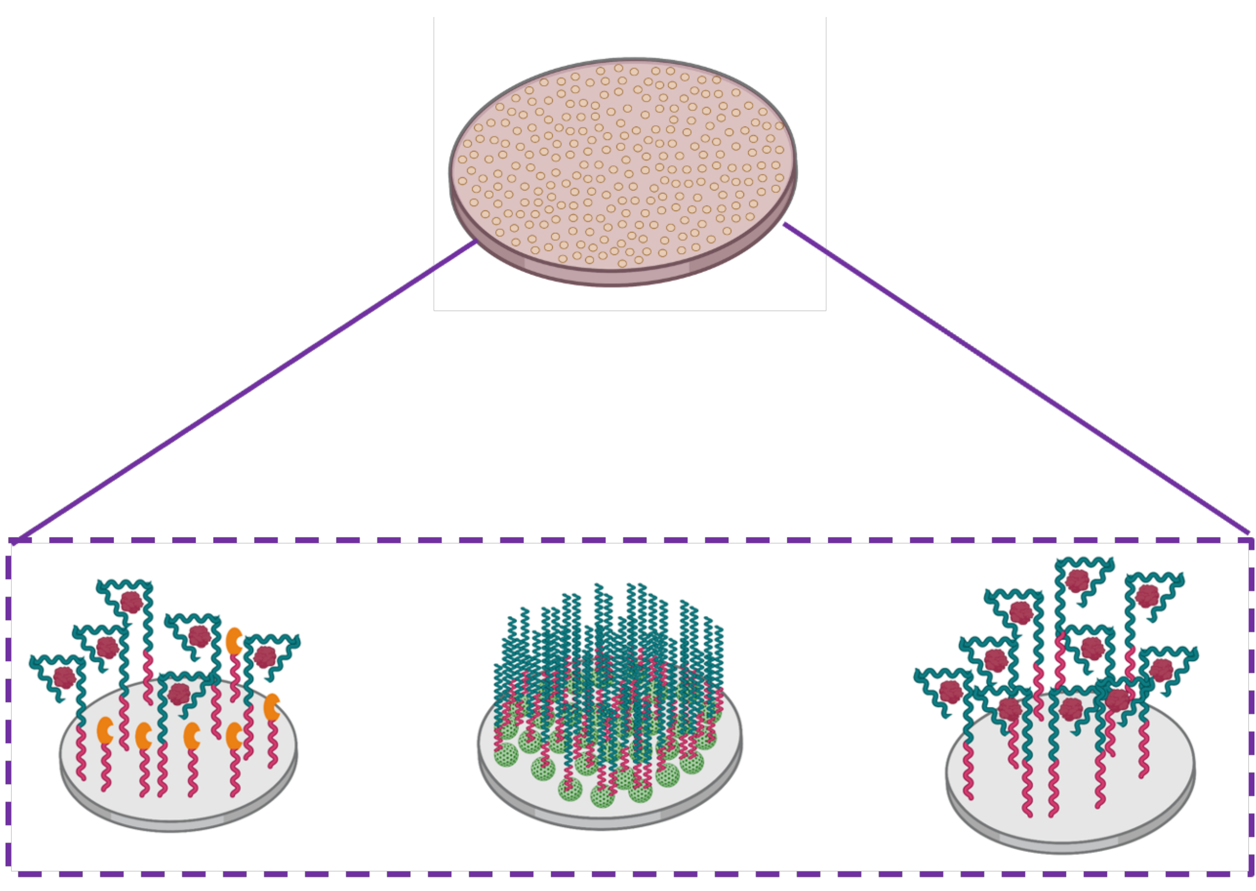
All these activities resulted in a perception that is often not seen in academia, and I hope this discussion served as an introduction or a spark to start talking about how to make graduate students be more excited about what they are learning are getting prepared for their future career interests. Students (especially the ones that want a career outside of academia) should maximize the use of professional development resources that their university provides as this will allow them to follow their passion and prepare them for their future career. Professors should support their

graduate students' passions so that they can have a fulfilling experience that can allow them to follow their dreams.

While research was a big part of my PhD, the activities and training that I developed outside the lab over the course of my PhD allowed me to become an effective leader and science communicator. This holistic training allowed me to get prepared for my future career. I was able to find a tenure-track academic job right out of graduate school, which are often very competitive, and could not have been successful it without the opportunities given to me that go beyond the benchtop work.

Chapter 6

Conclusions and Future Directions



In this chapter, we reflect on the usage of aptamer-functionalized membranes in the field and the potential future uses for this kind of technology.

6.1 Conclusions

Throughout the last five chapters, we have described a novel preparation method that incorporates the use of ultrafiltration membranes and aptamers for the selective capture of small molecule contaminants and macromolecules from water. We used a hydrophobic polymer PES as the main polymeric entity of our membrane, and we grafted polymethacrylic acid (pMAA) to add hydrophilicity and a point of attachment for an amine modified aptamer. We were able to optimize aptamer attachment as this is the most "expensive" component in our method. Furthermore, we were able to test a variety of aptamers of different nucleotide lengths to assess the versatility of this method.

Most interestingly, we were able to prove that this was a functional system by: (1) depleting the small-molecule of interest (bisphenol A, atrazine, or microcystin-LR) using the aptamer-functionalized membrane, (2) regenerating this membrane by subjecting the membrane to hot washes (60 ºC) and depleting the small molecule for multiple cycles and (3) removing multiple small-molecules and macromolecules synergistically from water by attaching three unique aptamers to the same membrane.

We anticipate that this technique will be generalizable for any small-molecule target, because of the current progress in SELEX development, especially in water-borne toxins and contaminants. Through the methods discussed, we have generated a potential point-of-use system that can be adaptable to community needs. We are encouraged by the results obtained thus far and are excited to move forward into further applications of these systems. We are enthusiastic about the use of aptamer-functionalized membranes in applications that go beyond the decontamination of water like concentration of important analytes or biosensing.

Lastly, apart from creating a useful method for the removal of small molecules from water, we also reflect on the graduate school path and how it can be more than just research to have a well-rounded experience and be prepared for long-term goals. The usage of this time to get as much professional development as possible is a characteristic that is not present in the majority of the

graduate student body and must be talked about for the benefit of the students, especially the ones that are interested in careers outside of academia.

6.2 Future Directions

Due of the malleability of the membrane, thickness can be reduced so that we can attach a single-layer nano-particle array that increases surface area and therefore aptamer attachment. Another exciting application is their use in the recovery of small-molecules of interest, which are often in low concentrations, and the membrane-aptamer scaffold can be used for the continuous recovery of small-molecules not only from water, but from biological matrices as well. In this section, we explain how we plan to implement our system to: (1) attach a single-layer nanoparticle system to the membranes to decrease the width of the membrane and offer more surface area. Due to the single-layer compacting design, the thickness of the membrane can be reduced to low micron levels, contrary to the previous membrane (mid-micron level thickness). This will greatly reduce the material usage and help ultimately fabricate a lighter and cost-effective efficient setup. (2) to bind and concentrate nutrients in order to increase their concentration. Such instances also include the recovery of small molecules and precious analytes from water (e.g. natural products for medicinal and therapeutic purposes), as well as pre-concentration of analytes that can be applicable to a wide range of biosensing applications.

6.2.1 Use of a Single-Layer Nanoparticle System

With less than one percent of water being fresh, we are rapidly depleting the sources of clean drinking water and we are projected to face a serious scarcity as soon as 30 years from now.[1] Due to this alarming reason, the investigation into decontaminating water has rapidly increased in the past few decades.[2] Scientists and engineers have identified various methods for water purification, but these methods have to strike a balance between efficiency, sustainability, and

financial viability.[3] Unfortunately, the most common methods for purifying water require the use of several components with sequential purification steps, which are economically not feasible for developing countries to implement.[4] Methods for water decontamination such as coagulation, sedimentation, or physical and chemical inactivation can be quite expensive and taxing on our resources because they rely on the heavy use of machinery, electricity, and pressure.[5] For these reasons, there is a pressing need to focus on the development of user-friendly devices that are affordable, sustainable, and effective at the same time.

To accomplish this, we need a system that can purify most contaminants in “one-go”. Synthetic membranes offer a facile solution to this problem, as they have proven to be more efficient, are relatively cheaper, and more user-friendly than other water purification methods.[6] Recent advances have shown that point-of-use devices (e.g. LifeStraw) are able to remove most macromolecular contaminants.[7] However, they suffer from several limitations including but not restricted to their inability to filter out small molecule contaminants and toxins. Small molecule contaminants and toxins are typically ubiquitously present in a given contaminated water source and can have harmful effects in the human body.[8]

Aptamers are single stranded DNA that have high affinity to specific molecules of interest, thus serving as a great platform for molecular detection and screening. These DNA structures adopt a specific secondary structure that then allows them to bind to a specific molecule of interest with non-covalent forces: hydrogen bonds, pi stacking, electrostatic forces, etc.[9] By enabling attachment of these aptamers to the membrane’s surface, we will be able to pave the way for tandem removal of small-molecule and macromolecular contaminants, toxins, and microorganisms from water in a user-friendly manner. I propose the functionalization of aptamers onto the membrane, thereby increasing selectivity and affinity to select contaminants.

We envision to explore the introduction a more delicate structural design with a highly ordered single layer of deposition of silica nanoparticles. Silica nanoparticles are known for their lack of cytotoxicity, pH resistance, and are chemically amenable and easy to synthesize.[10] The highly compacted particle layer will allow us to form a highly organized porous structure (instead of the random porous alignment in our previous approach), thus endowing a very high surface area that can increase the quantity of DNA aptamers that will attach to the membrane.[11] We are currently examining the attachment and functionalization strategy of the nanoparticles. We are preparing amino-modified silica particles that will be able to covalently bind onto the membrane (**Figure 6.1**). Due to the single-layer compacting design, the thickness of the membrane can be reduced to low micron levels, contrary to the previous membrane (mid-micron level thickness). This will greatly reduce the material usage and help ultimately fabricate a lighter and cost-effective efficient

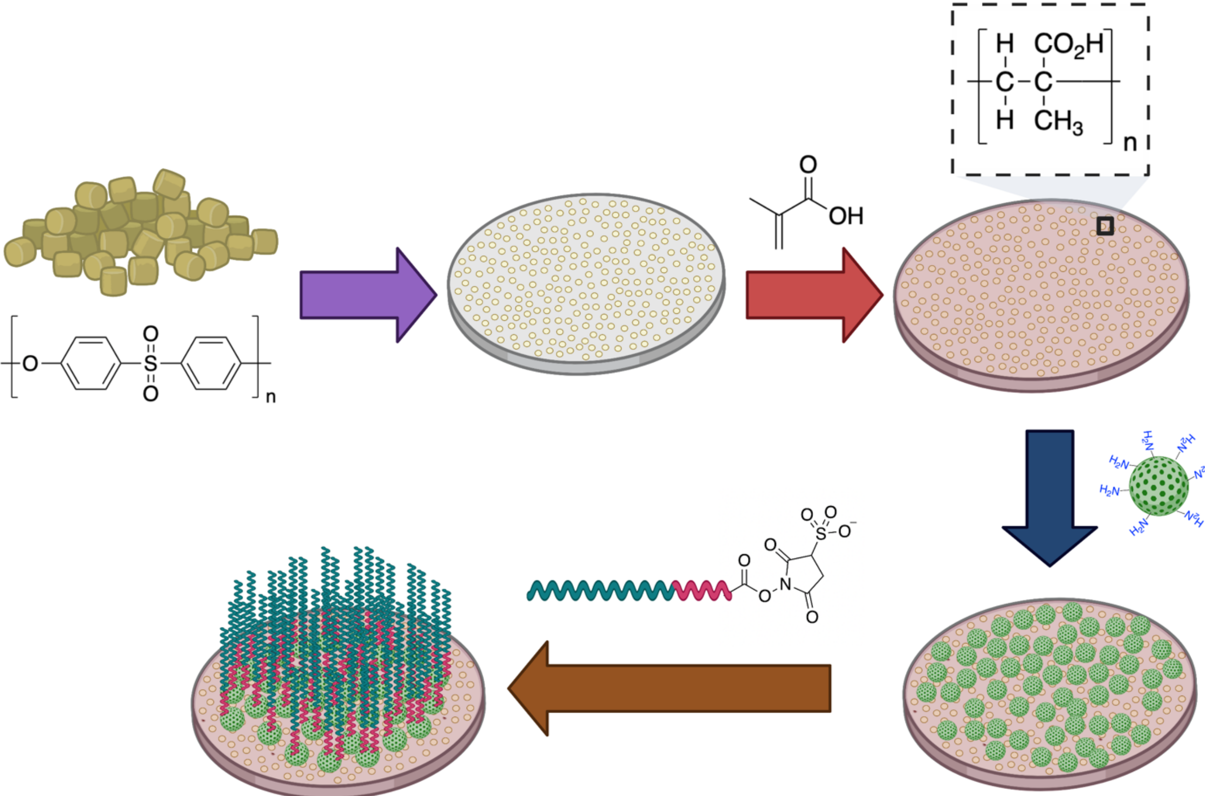


Figure 6.1: General scheme for the formation of single-layer silica nanoparticle system with aptamers for enhanced small-molecule depletion

setup. We hypothesize that a densely packed aptamer-functionalized membrane will be able to rapidly bind and detect such low concentrations in water, and recovery will be possible by a simple denaturation of the aptamer. This is another unique feature of aptamers, as they can detect and bind to their target in low concentrations.

6.2.2 Recovery of Small-Molecules using Aptamer-Functionalized Membranes

Water decontamination serves a necessary role in multiple processes aside from consumption. Such instances also include the recovery of small molecules and precious analytes from water (e.g. natural products for medicinal and therapeutic purposes).[12] Current techniques such as liquid-solid chromatographic techniques, extraction methods, preparative gas chromatography, etc. for isolating natural products from water are low-yielding because the presence of these compounds (concentrations) are inherently low.[13] We envisaged that our water purification strategy would be able to rapidly sequester multiple small-molecule components, and the scope can be eventually expanded to recover small molecules. This is because of the ability of sequestration of the aptamers. We can explore the concentration of precious small molecules. Apart from the precious small-molecules from water sources, we envisioned that the gap also happens in the body. There are small-molecules within our body that are inherently in low concentrations, because of this, the necessity of concentrating these molecules for further biosensing is important.[14] Current methods either require big concentrations of serum (from the body), and that is not feasible. For this reason and to minimize components and point-of-use care, we decided to test if our membranes would aid in the concentration of small-molecules of interest. To achieve this, we would have to attach aptamers to an ultrafiltration membrane and be able to sequester a small molecule in low concentration (**Figure 6.2**). We hypothesize this can be relatively easy because you can filter the small-molecule filtrate (coming from the solution passing through the membrane) repeatedly until you get the desired sequestration. Furthermore, we can decrease the membrane size to fit a 96-well plate for fluorescence or colorimetric experiments.

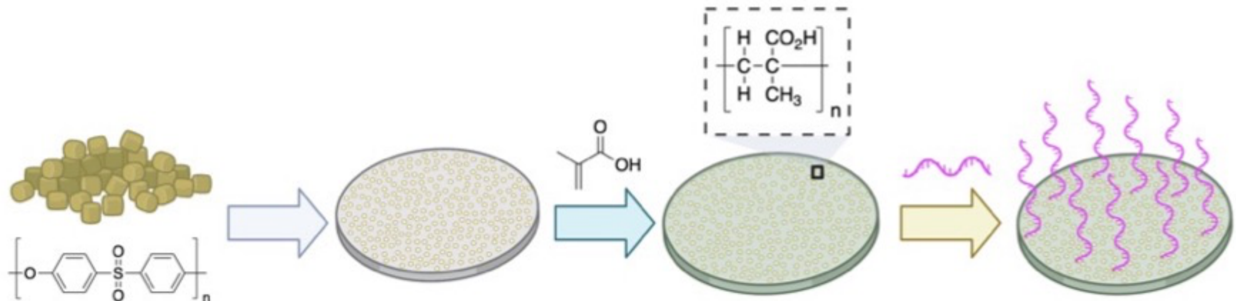


Figure 6.2: General scheme for the preparation of small-molecule concentration membranes.

Because the membrane is really malleable, the reduction in size of the membrane would be relatively easy.

Current methods for small molecule sequestration are constrained by the use of expensive machinery and methods which make it difficult to be translated to resource limited environments, an area where they are currently needed. Biosensors have shown great promise for use as low-cost, point-of-care diagnostic tools, as they can harness nature's diverse repertoire of sensing molecules to detect clinically relevant biomarkers and transduce the signal into an easily detectable output.[15] Cell-free sensor systems (CFS) require small volumes of sample, can be freeze-dried and stored at ambient temperature, have no cell membranes to interfere with transport of the target molecule, and can produce distinct colored outputs. One of the drawbacks of CFS, is that it does not necessarily enable detection of clinically relevant concentrations. [16] We will overcome this by utilizing aptamer-functionalized membranes to concentrate the analyte and detect clinically relevant levels of key nutrients (e.g. B_{12}). Together this research can establish a novel and field-friendly approach for quantifying clinically relevant levels of key nutrients and expand the current capabilities of aptamer-functionalized membranes, significantly benefitting both basic science and human health.

6.3 References

1. Rijsberman, F. R., Water scarcity: Fact or fiction? *Agricultural Water Management* **2006,** *80* (1), 5-22.

2. Xiang, Q.; Fan, L.; Li, Y.; Dong, S.; Li, K.; Bai, Y., A review on recent advances in plasma-activated water for food safety: current applications and future trends. *Critical Reviews in Food Science and Nutrition* **2020**, 1-20.

3. Ying, Y.; Ying, W.; Li, Q.; Meng, D.; Ren, G.; Yan, R.; Peng, X., Recent advances of nanomaterial-based membrane for water purification. *Applied Materials Today* **2017,** *7*, 144-158.

4. Bouwer, H., Integrated water management: emerging issues and challenges. *Agricultural Water Management* **2000,** *45* (3), 217-228.

5. Folens, K.; Huysman, S.; Van Hulle, S.; Du Laing, G., Chemical and economic optimization of the coagulation-flocculation process for silver removal and recovery from industrial wastewater. *Separation and Purification Technology* **2017,** *179*, 145-151.

6. Judd, S. J., Membrane technology costs and me. *Water Research* **2017,** *122*, 1-9.

7. Khan, S. T.; Malik, A., Engineered nanomaterials for water decontamination and purification: From lab to products. *Journal of Hazardous Materials* **2019,** *363*, 295-308.

8. Rathi, B. S.; Kumar, P. S.; Show, P.-L., A review on effective removal of emerging contaminants from aquatic systems: Current trends and scope for further research. *Journal of Hazardous Materials* **2021,** *409*, 124413.

9. Carnerero, J. M.; Jimenez-Ruiz, A.; Castillo, P. M.; Prado-Gotor, R., Covalent and Non-Covalent DNA–Gold-Nanoparticle Interactions: New Avenues of Research. *ChemPhysChem* **2017,** *18* (1), 17-33.

10. Wang, L.; Zhao, W.; Tan, W., Bioconjugated silica nanoparticles: Development and applications. *Nano Research* **2008,** *1* (2), 99-115.

11. Moghal, J.; Kobler, J.; Sauer, J.; Best, J.; Gardener, M.; Watt, A. A. R.; Wakefield, G., High-Performance, Single-Layer Antireflective Optical Coatings Comprising Mesoporous Silica Nanoparticles. *ACS Applied Materials & Interfaces* **2012,** *4* (2), 854-859.

12. Chemat, F.; Abert-Vian, M.; Fabiano-Tixier, A. S.; Strube, J.; Uhlenbrock, L.; Gunjevic, V.; Cravotto, G., Green extraction of natural products. Origins, current status, and future challenges. *TrAC Trends in Analytical Chemistry* **2019,** *118*, 248-263.

13. Visht, S.; Chaturvedi, S., Isolation of natural products. *Journal of Current Pharma Research* **2012,** *2* (3), 584.

14. Figueiredo-Filho, L. C. S.; Silva, T. A.; Vicentini, F. C.; Fatibello-Filho, O., Simultaneous voltammetric determination of dopamine and epinephrine in human body fluid samples using a glassy carbon electrode modified with nickel oxide nanoparticles and carbon nanotubes within a dihexadecylphosphate film. *Analyst* **2014,** *139* (11), 2842-2849.

15. Allen, L. H.; Miller, J. W.; de Groot, L.; Rosenberg, I. H.; Smith, A. D.; Refsum, H.; Raiten, D. J., Biomarkers of Nutrition for Development (BOND): Vitamin B-12 Review. *The Journal of nutrition* **2018,** *148* (suppl_4), 1995S-2027S.

16. McNerney, M. P.; Piorino, F.; Michel, C. L.; Styczynski, M. P., Active Analyte Import Improves the Dynamic Range and Sensitivity of a Vitamin B12 Biosensor. *ACS Synthetic Biology* **2020,** *9* (2), 402-411.