Small-Molecule Sequestration using Aptamer-Functionalized Membranes

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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 aptamer-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.

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.[1-5] Methods including coagulation, flocculation, sedimentation, and photon-based inactivation are capable of removing these contaminants from water.[6] 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. Thus, 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.[6-8] 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.[9,10] Small molecules can be effectively removed using membranes having smaller pore sizes, however this also increases production cost and the resources needed for use.[7] 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 (Figure 1). Aptamers are single-stranded nucleic acids that are capable of binding to a target molecule with high affinity and specificity.[11,12] 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.[13,14] 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.

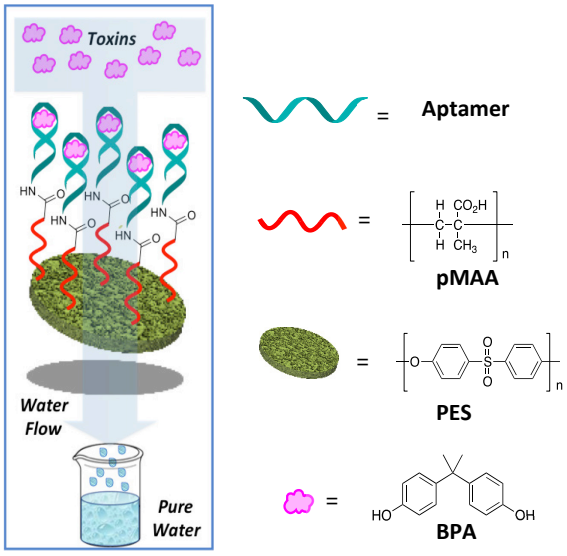

Figure 1. Depletion of small-molecule water contaminants using aptamer-functionalized membranes.

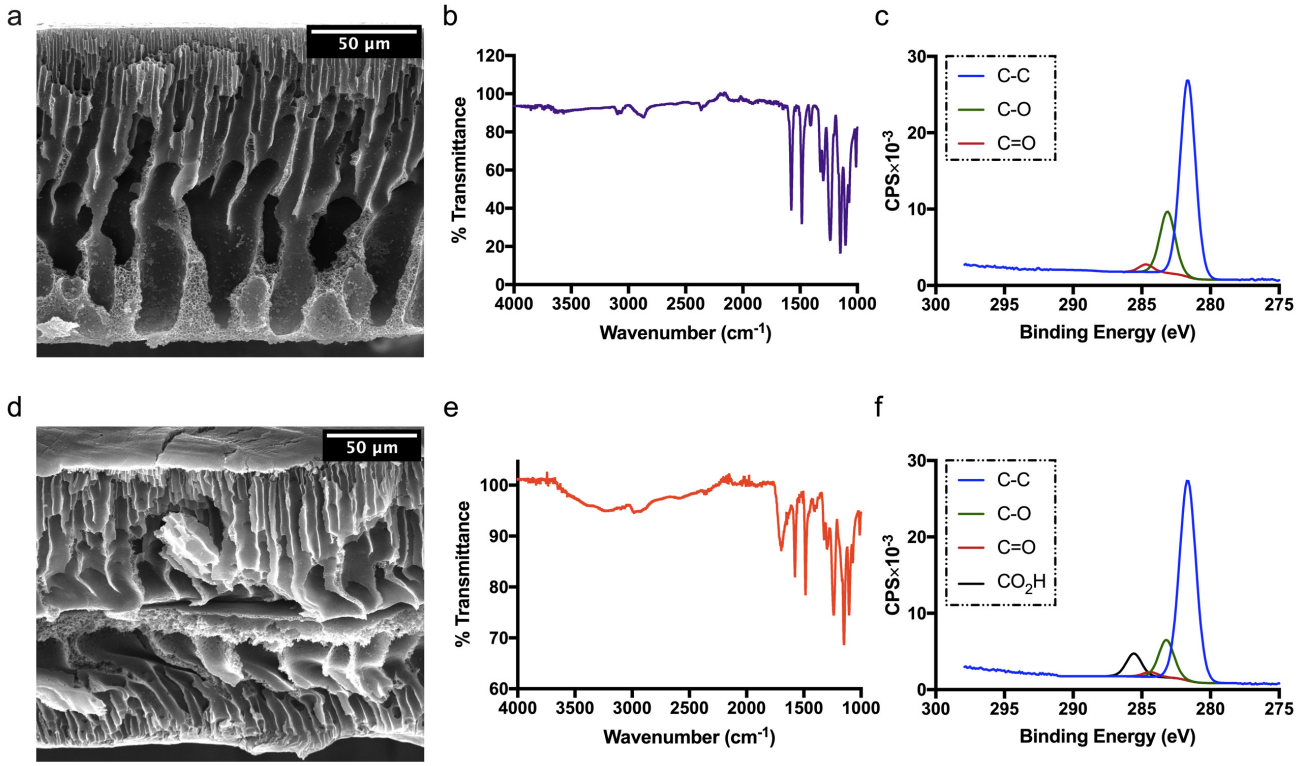

Figure 2. Characterization of grafted membranes. (a) SEM image of ungrafted membrane showing the formation of large macro-voids and the pores of an ultrafiltration membrane. (b) IR spectrum of ungrafted membrane. (c) XPS spectrum of ungrafted membrane. (d) SEM image showing similar structure after grafting. (e) IR spectrum of the grafted membrane showing the addition of carboxylic acid functional groups. (f) XPS spectrum of the grafted membrane showing the addition of carboxyl groups with a characteristic binding energy of 286 eV.

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,[13] diclofenac,[13] and ochratoxin A[15] from water. However, these approaches require complex preparation techniques, have low flow rates, and lack the ability to simultaneously remove larger contaminants when desired.[7]

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.[16,17] 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.[18-21] 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.

To generate the aptamer-functionalized ultrafiltration membranes, we utilized polyether sulfone (PES) grafted with polymethacrylic acid (pMAA).[7,22] The dual hydrophobic/hydrophilic nature of this membrane structure has been shown to provide favorable selectivity-permeability characteristics,[23] and the carboxylic acid functional groups of the pMAA offer a convenient point-of-attachment for amine-functionalized aptamer molecules. Following a modified procedure of Shi and coworkers,[24] PES membranes were prepared using the phase inversion process, then grafted with pMAA. Membranes were characterized by infrared spectroscopy (IR), X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM) (Figure 2). Results indicate that membrane grafting can be completed with no deleterious effects on pore size and structure.

In addition to serving as a useful point of attachment for the aptamers, the pMAA serves an important function by aiding in water transport and reducing fouling by bacteria and other molecules.[24-25] Given the large abundance of carboxylic acid functional groups and the anionic nature of the DNA to be conjugated, we hypothesized that aptamer functionalization would not detract from the desired characteristics imparted by the pMAA.[26] To investigate the reactivity of the carboxyl groups on the membrane and optimize conjugation conditions, aminofluorescein was initially used as an aptamer surrogate. Using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and hydroxybenzotriazole (HOBt) as coupling reagents, and fluorescence readout for quantification, we achieved an aminofluorescein functionalization yield of 12.9 ± 0.9 µmol per milligram of membrane. In a control experiment using an ungrafted membrane subjected to the same coupling reaction conditions, we observed no functionalization (Figure S10).

We then tested membrane functionalization using an amine-modified aptamer, which has been previously used for BPA detection.[27] We observed no functionalization with the conditions used for aminofluorescein attachment, and thus carried out further optimization studies. We observed that conjugation using *sulfo*-NHS and EDC under conditions described by Li et al. [28] produced the best results over other conditions such as EDC/HOBt/DIPEA (N,N-Diisopropylethylamine) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM), which resulted in no detectable reaction. As a result, the membrane was activated with the *sulfo*-NHS and EDC coupling reagents and then submerged in a solution of the amine-modified ssDNA in 3-(N-morpholino)propanesulfonic acid (MOPS) buffer. In order to establish and standardize our functionalization protocol, ssDNA loading was initially measured by dissolving the membrane in DMSO and quantifying fluorescence, using an appropriate calibration curve (Figures S14-S15). While highly accurate, this characterization approach is destructive to the membranes, and thus after reaction optimization we switched to monitoring the quantity of DNA remaining in the supernatant as an indicator of reaction efficiency. Importantly, control experiments with ungrafted membranes and subsequent washing of the membrane showed that depletion of DNA from the supernatant is due to covalent attachment on the membrane (Figure S13). Using our optimized reaction conditions, we observed that 43% of the anti-BPA aptamer[27] was conjugated to the membrane (Figure 3).

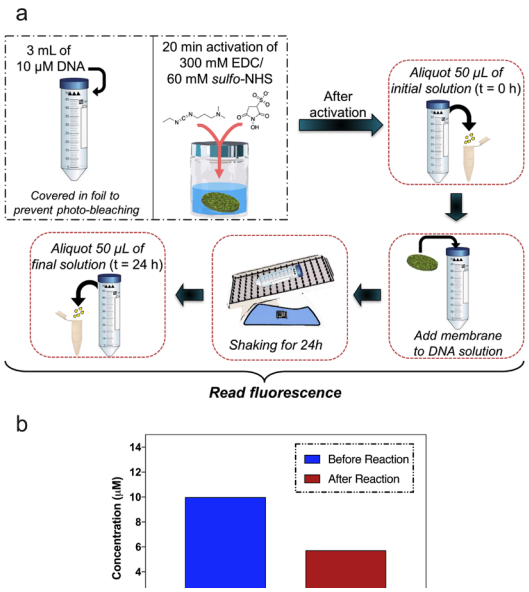

Figure 3. (a) Membrane functionalization with an anti-BPA aptamer. (b) The decrease in DNA concentration in the supernatant was used as a metric to quantify the amount of aptamer attached to the membrane. (c) Sequence of anti-BPA aptamer used for membrane functionalization.

Following the generation of the aptamer-functionalized membranes, we first tested BPA removal efficiency by preparing a feed solution of Milli-Q water spiked with 200 nM BPA, a concentration that is routinely found in water sources.[29] We analyzed BPA removal efficiency by quantifying the permeate concentration using HPLC. Concentrations were calculated by comparison to a calibration curve generated using samples having known concentrations of BPA. As shown in the breakthrough curve in Figure 4, we found that a circular 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

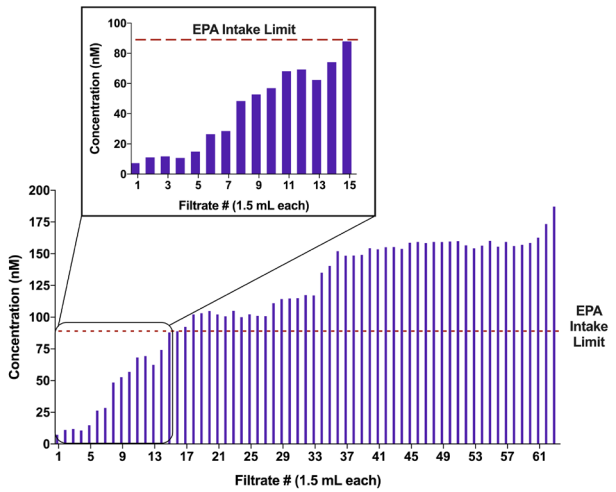

Figure 4. Breakthrough curve depicting BPA depletion of Milli-Q water as a function of the volume of water filtered, using a 200 nM BPA feed solution. Parameters: 1 bar of pressure, 1.5 mL filtrate collection volumes, Amicon® Stirred Cell 50 mL apparatus.

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 Environmental Protection Agency (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 (K_d = 8 nM)[30], 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. We also highlight that while this initial demonstration represents a relatively small volume of water, the membranes used in practical applications would be significantly larger and thereby having higher removal capacity. For example, current dimensions of personal-use water purification membranes are 23 cm x 23 cm, which allows for 34 of our membranes to fit in an equivalent area size, and thus enable the purification of ~850 mL of water. To ensure that contaminant depletion was attributable to the aptamer and not to non-specific adsorption, control experiments were carried out using a membrane grafted with pMAA, but having no aptamer attached, and we observed no detectable depletion of BPA from the water. (Figure S21).

We further evaluated the specificity of the membrane-bound BPA aptamer by exposing the membrane to Milli-Q water feed solutions spiked with diethylstilbestrol, a BPA analog, or 4-chlorophenol, a common phenolic contaminant found in water. We observed no change in the permeate concentration after multiple rounds of filtration, suggesting that attachment to the membrane does not interfere with aptamer selectivity and demonstrating that the aptamer-functionalized membranes are able to specifically sequester the BPA target (Figure S23).[25]

We were curious to explore regeneration of the membranes, as this would enable multiple uses, and also allow recovery of valuable small-molecule analytes. The retentate of the same membrane from the BPA depletion experiment described above was washed away with water at 65 °C, as this temperature disrupts the aptamer structure without damaging the membrane.[15] 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. (Figures S24-S26).

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 (Figure S28). 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 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 5). We performed a qualitative analysis on the unfiltered and filtered lake water to observe the ability of the functionalized membrane to remove larger contaminants (Figure S28a). The filtration process was able to remove particulates and other large matter from the sample, as noted by the visible change in the water.

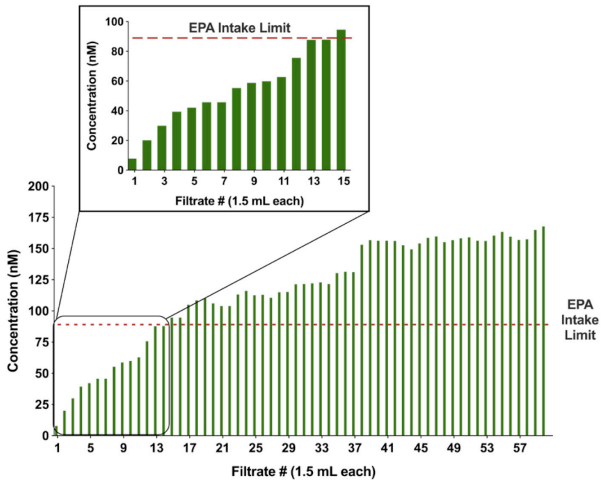

Figure 5: Breakthrough curve showing BPA depletion of lake water as a function of the volume of water filtered, using a 200 nM BPA feed solution. Parameters: 1 bar of pressure, 1.5 mL filtrate collection volumes, Amicon® Stirred Cell 50 mL apparatus.

The ability to sequester specific small molecules of interest would enable the removal of dangerous contaminants from the environment or the recovery of precious compounds such as natural products. Aptamers offer a promising solution to this challenge, as 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. We envision the future expansion of this method to enable the sequestration of a diverse range of analytes.

ASSOCIATED CONTENT

Supporting Information. General experimental protocols, synthetic protocols, and characterization data for membrane formation, grafting, functionalization, and BPA depletion. This material is available free of charge via the Internet at http://pubs.acs.org.

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