Progress Report: Cost-efficient detection of endocrine-disrupting compounds in drinking water

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Problem:

New Mexico has recently increased its utilization of surface water resources[1] to meet increasing water demands and preserve heavily taxed aquifers. While this may have some advantages to water conservation it does increase the potential for contamination of drinking water by certain harmful substances. Endocrine-disrupting compounds (EDCs) are one such class of contaminants which are known to be widespread in urban drinking water, especially those with surface water as their source.[2] EDCs are organic xenobiotic chemicals which disrupt hormonal signaling, and some (such as bisphenol-A or BPA) are known to have significant detrimental impacts on mammalian health and development at concentrations as low as several parts per billion.[3] Since they are still active at such low concentrations, harmful amounts of EDCs are often difficult to detect, and advanced methods such as gas chromatography-mass spectrometry (GC-MS) are presently needed to quantify these low concentrations.[2,3] However, this is undesirable because of the high cost and time-investment of these procedures.

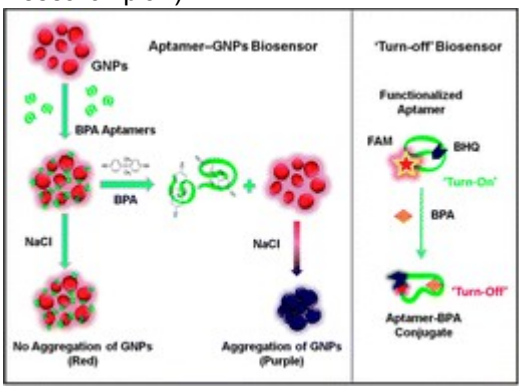
Objectives:

To develop a method to cheaply and quickly detect one of the most common EDCs: BPA.

Primary Research Plan:

- Employ a previously developed aptamer[4] – or sequence of nucleic acids selected to bind a target – to recognize BPA and produce a fluorescent "turn off" sensor.[5]

  Fig. 1 Schematic showing mechanism of a "turn off" sensor from Ragavan, *et. al.* as well as the mechanism of another popular sensing method employing gold nanoparticles (GNPs, see secondary research plan).



- Immobilize the aptamer "turn off" sensor onto a surface using the interaction of biotin and streptavidin to create an array of sensing molecules.[6]
  - Construct calibration curves for sensor arrays to determine the accuracy of each and reproducibility between sensor batches.

- Test re-usability of sensor by developing denaturing/refolding program for immobilized aptamers.

Secondary Research Plan:

Optional objectives which serve the purpose of this project include:

- Development of alternative sensor constructs using the BPA aptamer which would further decrease costs or increase ease of use, especially if the previously proposed mechanism is proven ineffectual.
  - This includes the use of methods such as the gold nanoparticle (GNP) based colorimetric sensor shown in Figure 1.
- Measurement of BPA concentrations in local surface water sources such as the Rio Grande or in municipal drinking water in Socorro, NM.

Results:

Primary Research Objectives:

A BPA aptamer molecule with a fluorescent tag (fluorescein adenine mononucleotide or FAM) at the 3' end and a fluorescent quencher (blackhole quencher or BHQ) at the 5' end was used in all reported upon experiments. Aptamer solutions were prepared and various concentrations of BPA added to them, all according to the methods of Ragavan, *et. al.* The fluorescence of these solutions was then measured using both a TD700 fluorimeter and a Victor 2 Wallac 1420 plate reader.

Figure 2. Measurements made using the TD700 fluorimeter. Fluorescence change is calculated by subtracting the value, in arbitrary "fluorescence units (FSU)", of a blank aptamer solution from those with added BPA.

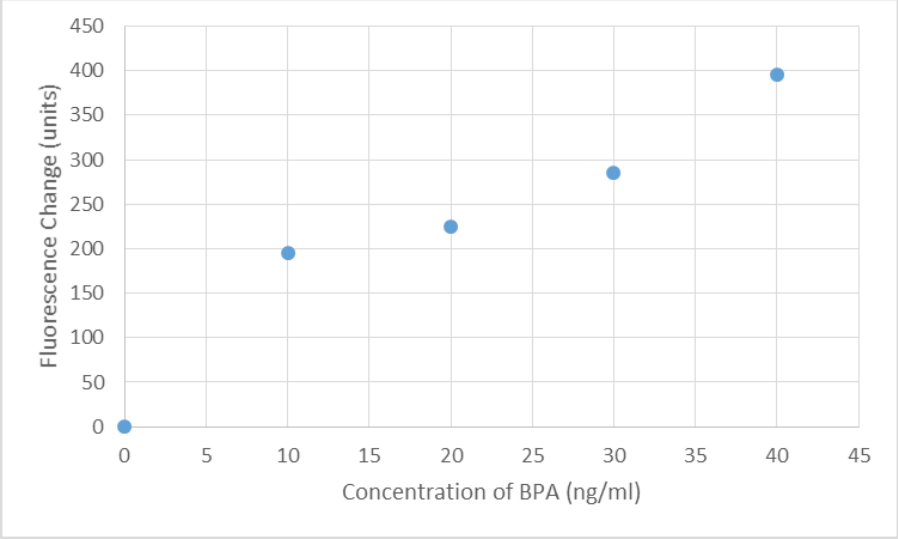
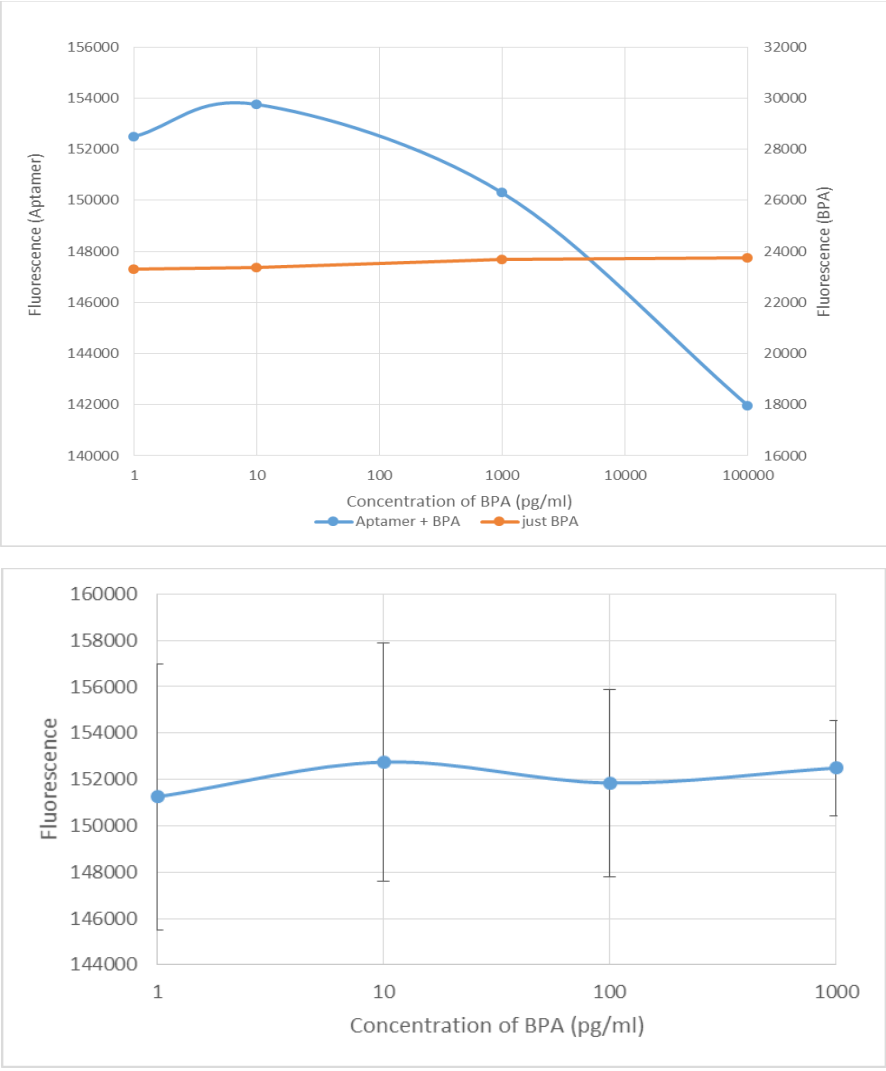


Figure 2 shows the results obtained from the TD700 fluorimeter, which is in this case the most simplistic instrument used. Fluorescence intensity was seen to increase upon addition of increasing concentrations of the aptamer, the opposite of what would be expected. The possibility that BPA itself or the DMSO solvent it was dissolved in were accounting for this increase was explored and ruled out by several experiments. These strange results along with the

lack of precision of the TD700 (solution fluorescence in all cases increased by hundreds of FSU's after several minutes in the instruments) led to this method being abandoned.

Consequently a more advanced fluorescence instrument was now chosen: the Perkin Elmer Victor 2 Wallac 1420 fluorimeter plate reader, which is capable of measuring several wells at once with chosen fluorescence emission and excitation wavelengths. Representative data obtained from this instrument are shown in figure 3.

Figure 3. Top: Initial and promising data obtained from the Wallac 1420. Fluorescence is in arbitrary units. Data for both aptamer and BPA combined and BPA alone are shown. Bottom: An average of all data collected using the standard protocol.[5] Data are shown only for BPA and aptamer combined in this case, and error bars represent the standard deviation of each set of measurements.



The top graph in figure 3 shows the initial promising data obtained from the Wallac 1420, with a downward and regular slope that would be expected of a "turn off" sensor. The flat trend in the case of BPA only also eliminates the potential that this is a confounding variable. A serious problem with these data, however, is that a low concentration samples (~1 pg/ml) has a fluorescence value similar to that of a 100 pg/ml BPA sample. This completely removes the potential utility of these particular data in predicting BPA concentrations in an unknown sample.

A further problem with this method is found in the bottom chart in figure 3, which shows compiled data from 12 experimental runs and a combined 26 replicates of each concentration. From this greater body of data it is apparent that virtually no trend can be observed in the changes in fluorescence with BPA concentration under the conditions used, especially when the values are compared to their standard errors (inset bars). There are several possibilities for why such large variability and lack of predictive power are observed:

- Original research by Ragavan *et. al*,[5] which this work was based upon, was overstated or incompletely described. Small facets of the protocol as reported may be important to the success of this method were not reported explicitly enough to be repeated.
- The instrumentation employed in our investigations so far is not sensitive or precise enough to detect the pertinent changes in fluorescence. This is despite the use of a more sensitive analytical fluorimeter, the Shimadzu RF-5301PC (data not shown).

Regardless of which of these factors is responsible for the poor data quality presented above it is clear that the methods employed so far are insufficient. While none of these attempts has been successful in detecting BPA, other factors that have already been varied in an attempt to make this method function properly include plate color, materials used in storage of stock solutions, placement of individual samples on the plate, instrument settings, addition of fluorescence enhancing/quenching gold nanoparticles, and presence/absence of BPA aptamer binding buffer and other salts.[4]

Secondary Research Objectives:

With the dismal results produced using the original approach we began investigating the possibility of using a colorimetric gold nanoparticle (GNP)-based sensor. The principle of such a sensor is explained in figure 1. The fundamental idea is that the binding of the aptamer to a target, in this case BPA, results in a color change in a GNP solution from red to blue. Investigations into this area were conducted based upon the protocols of Zhanlong *et. al.*[7] but no tractable results were obtained despite exploring variations in this reported protocol and efforts to synthesize GNPs of a precise size.

Conclusions:

A careful investigation of the capabilities of cost-effective aptamer-based sensing methods to detect the EDC BPA was conducted, primarily founded upon two research articles.[5,7] However, we were not able to replicate the results of either of these reports. While this may be due to some inherent failure(s) in the laboratory methods employed in this case, this still indicates that such sensing methods are not as robust as would be hoped for a economical approach to tackle the problem of small organic molecule contamination in drinking water sources. Altogether new sensing methods - or variations on these same protocols – may yet yield cheap and durable methods for detecting these contaminants with aptamers in a industrial or commercial setting, but we have not found that this to currently be viable.

Potential Benefits:

If successful, the immobilization of the functionalized aptamer into a microarray would have allowed for multiple reuses of each sensor. The final materials cost of each microarray sensor

would be approximately 60 dollars using even the most expensive aptamer molecule, which is less than a single GC-MS analysis not including technician time, and less than half as much as a high-resolution version of the same analysis. If such a microarray were to be reusable this would further attenuate costs. Aptamers that bind other EDCs could also be added to different areas on the same microarray, allowing for simultaneous measurement of several problematic compounds at once. However instrumentation would still be required to make these measurements, and as such the colorimetric GNP-based method would be preferred for ease of use. Although the GNP-based method also appears to involve expensive materials, each test would utilize approximately only $12 worth of materials. However this method would also lack the ability to measure several compounds at once. While we have not found these methods to be viable in our own investigation, the above discussed merits are certainly sufficient to warrant further research in this area.

An economical measurement method for BPA and other EDCs in drinking water could provide treatment facilities in many areas with the capability to more effectively regulate the levels of these harmful contaminants in their produced water. If this method is widely adopted it may also facilitate legislation which limits the amount of these compounds present in drinking water sources, a step which has yet to be taken by government agencies. The private well epidemiologist with the New Mexico Department of Health, Miriam Wamsley, has expressed her interest to our research group about developing the capability to easily measure these compounds in drinking water, and it is likely that other organizations outside of New Mexico would feel similarly.

Presentations Given:

Poster entitled: "Cost-efficient detection of endocrine-disrupting compounds in drinking water" at the New Mexico Water Conference, November 2014. An updated poster of the same title at the NM EPSCoR All Hands Meeting, April 2015.

Budget:

The table below shows the orginally allotted amounts and actual amounts spent during the project on materials and services.

Item	Cost per Unit	Proposed Number of Units	Allotted Amount	Amount Spent
Salary				
Max Baymiller	$10/hour	200 hours	$2000.00	$2,000.00
Reallocated	$10/hour	N/A	N/A	$2,682.61
Fringe Benefit				
2%	N/A	N/A	$40.00	$40.00
Travel				
NM WRRI Conference in Santa Fe	$250/round trip	1 round trip	$250.00	$138.28*
Supplies				
Functionalized aptamers	$142-288/20 nanomoles	200 nanomoles	$2150.00	$1139.11*
Streptavidin-coated slides and slide covers	$44-47/each	30 units	$1350.00	$0*
BPA analogs (BPB, BPS 4,4'-bisphenol and others)	$50-60/50 grams	4 different units of 50 grams	$210.00	$0*
Totals			$6000.00	$6,000.00

Two aptamers were purchased in this project, one being solely functionalized with 3' fluorescein (60 nanomoles) and the other both with 3' fluorescein and 5' black hole quencher (100 nanomoles). As difficulty was encountered in meeting the original project objectives, funds were diverted away from some materials to allow additional working time and a "*" denotes categories where funds were diverted to salary for such a purpose.)

Acknowledgements:

Dr. Frank Huang for advising on experimental design, and Tyler Pratt and Owen Brady for preliminary work on aptamers before the NM WRRI grant.

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