Capture and Removal of Ions in Ocean Water using Bacteria and Bacteriophage

Mina Akbary-Zheng, Rebecca Avileli, Logan Black, Tyler Black, Dewuni De Silva, Nimaya De Silva, Karen He, Emily Huynh, Mark Lea, Aroma Pageni, Nicolas Parra, Aidan Sander, Andy Sun, Michelle Wu, Alice Zhang, Wade Zhang

Catholic Central High School, Chinook High School, Lethbridge Collegiate Institute, Winston Churchill High School, University of Lethbridge, Lethbridge, Alberta, Canada

Reviewed on 28 April 2018; Accepted on 19 May 2018; Published on 22 October 2018

Access to clean drinking water is a critical issue for millions of people around the world. Water scarcity is expected to become increasingly common due to the effects of climate change, pollution, and mismanagement. By 2025, it is estimated that half of the world's population will live in water-stressed areas. Despite the abundance of water on Earth, less than 1% of water is accessible for human consumption due to physical inaccessibility, microbial contamination, and salinity. The desalination of ocean water is therefore appealing, but challenging due to the high energy requirements, inefficiency, infrastructure requirements, and low production volume. To address these issues, we propose the use of a system of engineered bacteria and bacteriophage—viruses that infect a bacteria and use its host to multiply—to capture and remove ions from solution using novel capsid composition and inducible precipitation. The utilization of biological systems and local infrastructure promises to provide a low-energy and high-yield desalination process to produce fresh water amenable for treatment and storage with limited waste. Increasing our capacity to utilize the water resources of the ocean will boost local resilience water scarcity and universally entrench the right to water.

Keywords: Desalination, Metal Ions, Bacteria, Bacteriophage, Virus, Synthetic Biology, Water

Mentors: Sydnee Calhoun, Chris Isaac, Dr. Laura Keffer-Wilkes, Erin Kelly, and Kristi Turton

Authors are listed in alphabetical order.
Please direct all correspondence to: sydnee.calhoun@uleth.ca

Watch a video introduction by the authors at https://bit.ly/2OsKf6d

Background

The accessibility of freshwater is a serious global issue that unites countries around the world. Although countries like Canada and Russia have large, potable freshwater reserves, 4 million people in Cape Town—South Africa's second largest metropolis—will experience a water shortage in early 2019 due to dwindling reserves (National Geographic (Nat Geo), 2018). Cape Town is not the only place in the world undergoing a lack of water; Tokyo, Cairo, and Miami are just a few examples of other cities struggling to maintain freshwater supply (Guest Contributor, 2015). This scarcity is attributed to a combination of climate change, the continuous growth

in world population size, and a deficit of investment for technological and infrastructural improvements to reliably obtain freshwater.

Approximately 97% of water on Earth is undrinkable for humans due to its saline or brackish state—and the drinkable 3% is declining due to the growing population. By the year 2050, worldwide freshwater consumption is predicted to rise by 55%, as estimated by the Organisation for Economic Co-operation and Development (OECD) (2012).This growing necessity for clean water demands an effective design for freshwater obtainment (World of Earth Science, 2003).

In order to acquire potable water, the dissolved salts in seawater at approximately 35,000 ppm must drop to a concentration of 200 ppm (Government of Canada, 1992). Current practices are inefficient, energy-consuming, and costly. For example, reverse osmosis prevents any molecules larger than water from going through, providing an excellent desalination and purification system. Unfortunately, reverse osmosis wastes four gallons of water for every gallon of fresh water produced (https://bit.ly/2DMomdG), requires high energy input—to create the needed pressure— is extremely time consuming, and tends to remove desirable minerals from water. Another existing method, distillation, also proves to be inefficient and unsustainable due to the high specific heat capacity of water and the resultant high energy required to boil the water. An effective method for water desalination can be created through the designing of a low-input, high-yield system with biology.

Systems Level

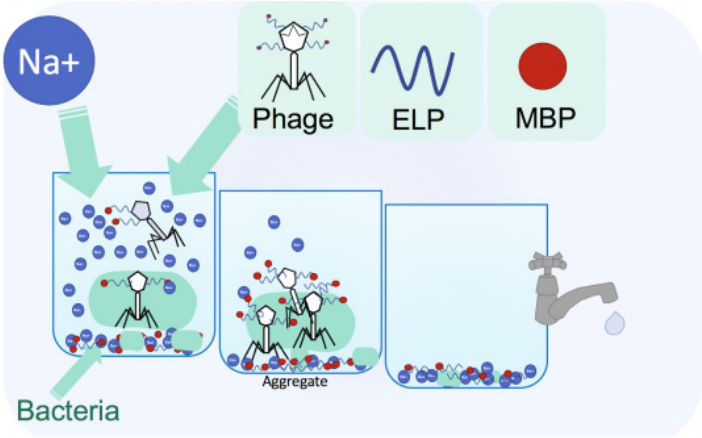

Figure 1. *Container model for desalination process. Our system contains a number of containers. In each container there is a salt reduction. The aggregate that contains the precipitated metal ions, phage, ELP, and bacteria will be removed. In the final container, the salt concentration will be at a level that is consumable for humans.*

To solve this issue, we propose a system composed of multiple cisterns filled with ocean water—35,000 ppm salt concentration—that contains engineered bacteriophage capable of salt binding (Figure 1). The phage will invade the host bacteria; this will produce an exponential numerical growth of these phages. This provides an efficient method of surface area increase for salt-binding action. As a result, the sodium concentration in the water will be reduced to 200 ppm. To enable the survival of the host bacteria for the phage in the high-salinity environment of ocean water, the bacteria will be engineered to possess salt stress-adaptive genes or will be naturally tolerant to halophilic conditions. Then, it will be cultured in a separate cistern and distributed among the other cisterns to reduce the sodium concentration for each successive step.

Phage display technology is used for a variety of purposes such as immunotherapy (Bazan, Calkosinski, & Gamain, 2012). Such technology has allowed experimenters to express foreign peptides onto coat proteins of phages. To express foreign molecules on a phage, a gene fragment is inserted into a gene corresponding to the surface protein of a bacteriophage. The desired protein then appears on the surface of the phage, which can then be utilized for a number of purposes.

To achieve this, an ion-binding protein that targets sodium ions or other metals of interest—silver, gold, etc.—will be attached to each phage. When bound to a protein, an elastin-like polymer (ELP) that is also attached to the phage will be used to induce the sodium-phage complex to flocculate. The flocculation caused by the ELP ensures that the desalinated water drains into the next cistern whilst the sodium phage complex remains to sediment at the bottom of the cistern for removal.

The lower maintenance, infrastructure, and energy costs of our system will effectively solve water crisis in countries with not only little freshwater, but countries that are near vast reservoirs of ocean or salt water. The system's ability is not only promising in the field of water desalination, but for metal extraction as well; ion-binding proteins could be used to remove heavy metals from tailing ponds or precious metals in mining apparatuses.

Device Level

Host Selection

When considering a potential host for our system, it is important that the host (1) can be parasitized by a phage amenable to engineering, (2) can survive in high salt concentrations, and (3) can resist temperature fluctuations with salt storage and aggregation. Accordingly, we have chosen to explore three bacteria as potential hosts: *Escherichia coli*, *Pelagibacter ubique*, and *Synechococcus elongatus* (Figure 2).

E. coli is a well-studied model organism with defined methods of genetic manipulation and is easily accessible in our lab. While *E. coli* has previously been identified as having low salt-tolerance, the 2012 University College London iGEM team found that by engineering the bacteria to express the protein IrrE, *E. coli* was able to survive in the oceanic salt concentrations of approximately 35,000 ppm NaCl (University College London, 2012).

Moreover, it has fast growth kinetics, where high-cell density cultures can be achieved easily (Rosano & Ceccarelli, 2014). Based on these characteristics, we will use *E.coli* for our proof of concept. The additional benefit of working with *E. coli* is that results obtained in the system will inform in silico modelling, allowing us to design other phage systems for different organisms.

After working with *E. coli*, we will proceed to *P. ubique*, a bacterium dominant in ocean surface waters. Its abundance—approximately 2.4 x 10^{28} microbes—will create sustainable deployment of water, due to the unnecessary addition of bacteria (Herlemann, Labrenz, Jurgens, Bertilsson, Waniek, & Anderson, 2011). Furthermore, it is one of the smallest free-living cells, and works efficiently. Modelling the system in *P. ubique* will demonstrate a possible method for our project. However, this bacterium requires the consumption of dead organic matter dissolved in ocean water to survive. Self-sustaining bacteria would be easier to work with, due to its ability to thrive without additional support.

Fortunately, the bacterium *S. elongatus* meets this criteria. This bacterium is photosynthetic and naturally replicates in limnic seawater (Weinbauer et al., 2011). The self-sustaining nature of this host provides dual benefits in terms of climate change and desalination. As more people populate Earth, carbon dioxide levels will rise accordingly, and the quantity of freshwater will decrease. *S. elongatus* will be able to use the greater carbon dioxide level to grow more abundantly, in addition to salt absorption. This cyanobacterium was also used by the 2015 Shanghai iGEM team in their project to absorb salt from salt water with biological membranes (SJTU-BioX, 2015). Furthermore, the presence of viruses promotes extensive growth for this bacteria (Weinbauer et al., 2011). *S. elongatus* will provide an ideal model for our project.

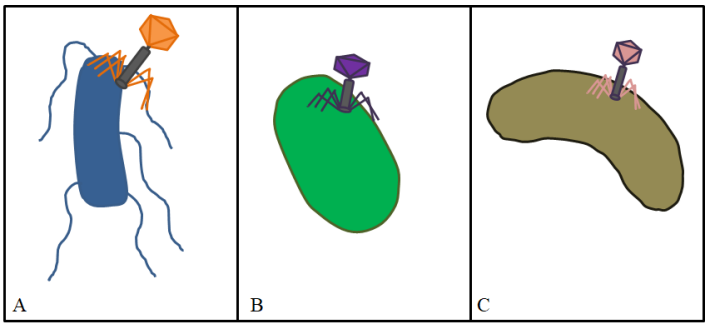

Figure 2. *Potential bacterial host-phage pairs for use in desalination. The hosts selected are Escherichia coli (A), Pelagibacter ubique (B), Synechoccus elongatus (C). The compatible phage propagated by each species of bacteria is T4 phage for E. coli, HTVC008M for P. ubique, and Syn5 phage for S. elongatus.*

In summary, the choice of host defines numerous properties of the system in terms of energy input, phage production, and efficiency of desalination. Our initial efforts in *E. coli* will inform our design of other host-phage systems, and alter the way in which our device works to capture and sequester ions in seawater.

Phage Selection

Accordingly, we have chosen phages that have a host range corresponding to our three potential hosts: *E. coli*, *P. ubique*, and *S. elongatus*. Through the utilization of phage display technology, we will attempt to express elastin-like polymers (ELPs) fused to metal binding proteins on the surfaces of the T4 phage for *E. coli*, HTVC008M for *P. ubique*, and Syn5 phage for *S. elongatus*.

The T4 bacteriophage belongs to the phage family Myoviridae and interacts with *E. coli* (Yap & Rossmann, n.d.). The capsid of this phage has been successfully modified to display peptides and protein domains on the surface. In past experiments, biologically active, full-length foreign proteins were displayed by fusion to SOC (small outer capsid protein) and HOC (highly antigenic outer capsid protein), accessory protein genes found on the T4 capsid surface (Yap & Rossmann, n.d.). In fact, a bipartite T4 SOC–HOC protein display system allowed two different proteins to be displayed on one T4 particle simultaneously (Ren & Black, 1998). The high abundance of these proteins—960 SOC and 160 HOC molecules per phage capsid—increases the efficiency with which potential salt and metal-binding proteins may function, once attached to this phage (Yap & Rossmann, n.d.).

HTVC008M is closely related to the Myoviridae family as reports have determined that it has an isometric head and a contractile tail structure (Zhao et al., 2013). Zhao et al. (2013) reported its capsid size to be approximately 84 nm. As this phage is similar to the T4 phage, it will allow for our data collected from the *E. coli* T4 phage system to be more transferable and applicable to this new host and phage.

The Syn5 cyanophage provides a way to demonstrate a real-life application of our project. Syn5 interacts with its host *S. elongatus*, and is found in abundant quantities in seawater. The phage's structure includes a tail and an icosahedral head (Suttle & Chan, 1993). In addition, the head structure of the cyanophage Syn5 includes a product of gene 58, a protein which is assembled onto the capsid lattice (Raytcheva, Haase-Pettingell, Piret, & King, 2014). Syn5 also includes a horn-like structure, which protrudes from a vertex opposite the tail. Through the use of immune-electron microscopy, Raytcheva, Haase-Pettingell, Piret, & King (2014) found that the horn structure includes two unique proteins—the products of viral genes 53 and 54—which do not seem to have any connection to the attachment of the phage to its host . Instead, the horn structure is hypothesized to help with

the attachment of the phage to foreign surfaces. This is highly beneficial to our project as it increases confidence in utilizing the Syn5 phage to express ELPs on the surface of the phage (Raytcheva, Haase-Pettingell, Piret, & King, 2014).

Precipitation by Elastin-like Polymer State Change

The purpose of elastin-like polymers (ELPs) is to remove the salt-laden phage from the solution. At a certain temperature, ELPs will aggregate and drop out of solution (Figure 3). This transition temperature (Tt) is influenced by factors such as salinity, concentration of ELP, the length of the ELP chain, and the variable amino acids in each monomer. We will alter the length and the concentration of ELP to achieve our goal Tt. We hypothesize that the ELP will have a Tt between 40°C and 50°C, as this will allow the ELP to remain in solution while maintaining the optimal efficiency of our chosen bacteria. The length and the Tt have an inverse relationship; when we increase the length of the ELP, the transition temperature decreases. Using this information, we intend to keep the guest residue the same while altering the length to achieve the goal Tt.

Parts Level

Elastin-like Polymer Design

With the transition temperature being 40°C for an ELP used for thioredoxin purification, the increase in length will significantly decrease the yield (Figure 3). The length was 90 repeats for the ELP (Meyer & Chilkoti, 2004). When aiming for a goal temperature between 40°C and 50°C, two ELP libraries at an ELP concentration of 25 μM in phosphate buffered saline (PBS) can be used, preferably ELP[V5] with 40 pentapeptides. This ELP will theoretically give us a transition temperature of approximately 50°C, while maintaining a relatively short length so that the yield remains high. Another possible ELP to use is ELP[V5A2G3] with 90 pentapeptides. This is not our preferred option as the ELP is longer, resulting in a lower yield; however, this ELP should also result in the preferred Tt (Meyer & Chilkoti, 2004).

To influence the characteristics of our ELP, we can experiment with the variable amino acid in each monomer (Figure 4). All ELPs contain the amino acid sequence Val-Pro-Gly-X-Gly, with X representing the variable amino acid. For our project, we want to use a hydrophobic amino acid, specifically valine, glycine, or alanine. By using a hydrophobic amino acid, we can destabilize the ELP, and it will more readily drop out of solution. There are several residues that work well with the precipitation of ELP; valine is found to be the most effective for protein aggregation. We will add standardized linker sequences to the N- and C-termini of the ELP to allow for easy fusion to other functional proteins. In our case, the capsid protein of the bacteriophage and the metal-binding peptide will be fused to either end of the ELP.

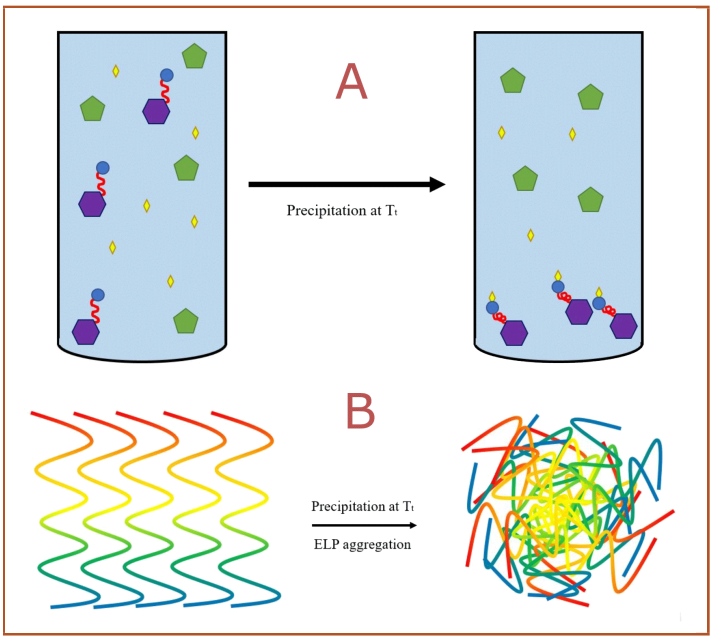

Figure 3. *Precipitation of ion-binding domains and phage-by-phase transition of elastin-like polymers. A) Sodium ions (yellow) or other metal ions B) ELPs are precipitated at the transition temperature (Tt) and attach to the metal-binding peptides (blue) which precipitate with the ELPs at the appropriate Tt.*

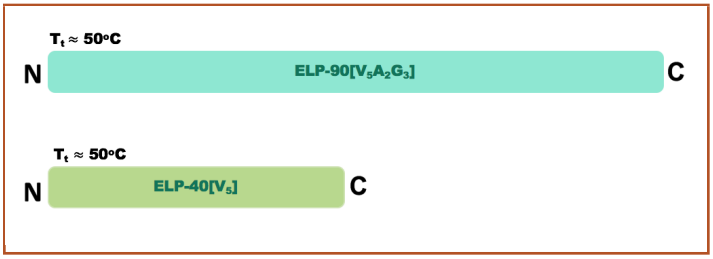

Figure 4. *Schematics of different variants of elastin-like polymers. The Tt for both ELPs is 50°C but the ELP that uses valine as the guest residue in the 40 pentapeptides is much shorter and therefore more efficient in precipitation as it uses less resources.*

Metal Binding Protein Candidates

Due to the requirement of sodium in many organisms, successful binding may be difficult to obtain; however, the binding of different metal ions may be easier. Examples of target metal ions are copper ions, cobalt ions, or iron ions. As these metals are used in many common products in our world today, such as copper and silver in phones, an efficient method of metal extraction from the water is an exciting prospect for the future industry

(Techradar, 2015). Even better would be the expression of both the water desalination and metal ion binding proteins in our system.

However, for our current project, metal binding proteins will only be used as a proof of concept for sodium ion binding. The following proteins are being considered:

PcoE (UniProt: Q47459)

Its high affinity to copper and small size makes Probable Copper Binding Protein (PcoE) a candidate. PcoE acts as a first line of defense against metal toxicity in *E. coli*; when attached to the phage, any metal ions will easily be absorbed by the protein. Firstly, PcoE sequesters soft metal ions with partial folding. It then transfers the sequestered Cu+ to PcoC for further detoxification. Using PcoE as a copper binding protein will allow large quantities of copper extraction when attached to the phage (Zimmerman et al., 2012).

SilE (UniProt: Q9Z4N3)

SilE is a relatively small monomer with a high affinity for silver binding, which clearly demonstrates its potential when fused to an ELP on the phage capsid or to the capsid itself, binding to large amounts of silver in the salt water solution. Since SilE is a protein that originates from a prokaryote, it will require no post translational modifications to function. Accordingly, it can effectively be produced in *E. coli* and incorporated into the phage.

RHCC (UniProt: Q54436)

Right-handed coiled coils (RHCC) are tetrabrachion stalk proteins from *Staphylothermus marinus*. RHCC is a good candidate for metal binding due to its small size and high affinity for metals such as gold. Additionally, the high thermostability of RHCC due to salt-bridge networks, surface hydrophobic interactions, and hydrophobic cavities ensure no alteration of its function at most temperatures (Stetefeld, Jenny, Schulthess, Landwehr, Engel, & Kammerer, 2000). Therefore, RHCC presents an attractive option for sequestering metal ions and salts.

CUP1 (UniProt: P0CX80)

CUP1 is a gene used in the yeast *Saccharomyces cerevisiae*, commonly known as baker's yeast. Upon the induction of copper ions, CUP1 encodes metallothioneins (MTs), metal binding polypeptides with low molecular mass. Using this as our metal binding protein will be beneficial, due to its tight chelating properties to copper ions. In addition, the longer length of CUP1 will increase the probability for proper attachment and function to the capsid protein, in comparison to using the sodium binding protein. CUP1 will most likely be used as our metal binding protein (Adamo, Lotti, Tamas, & Brocca, 2012).

Sodium Binding Di-, Tri-, and Tetrapeptides (Wang, Wesdemiotis, Kapota, & Ohanessian, 2007)

These proteins were validated with affinity for sodium. We chose this protein because it is a monomer and is relatively small. When fused to either an ELP or a capsid, these characteristics will enable it to effectively bind to sodium. The high sodium-binding affinity will enable it to bind to and bring with it large amounts of silver when eventually removed from solution. The Di-, Tri-, Tetrapeptide is a protein that originates from a prokaryote, so it will require no post-translational modifications to function. Accordingly, it can effectively be produced in *E. coli* and be incorporated into the phage. We will use this protein for sodium flocculation after testing our system with one of the above metal ion binding proteins.

In conclusion, the usage of metal binding proteins are ideal for multiple reasons. Firstly, they provide us with a proof of concept; when metals such as copper bind in solution, a colorimetric assay will easily demonstrate its success. In contrast, it is difficult to analyze sodium binding, as sodium in solution is colourless. Secondly, metal binding proteins—such as CUP1—bind much more readily to the capsid protein in comparison to the sodium di-, tri-, and tetrapeptides. This is due to the fact that metal binding proteins are longer than these peptides, increasing the likelihood for their success. Thirdly, the modularity of our system in presence of these metal binding proteins provide us with a variety of useful applications, as previously mentioned with the production of phones. Another application includes the oil sands industry. Current oil sands extraction demands large quantities of freshwater to separate the heavy crude oil from clay and sand—about three to four barrels of new water for every barrel of oil (Government of Canada, 2015). However, implementing our system with the current purification system will allow the extraction of metals with only the use of synthetic biology, reducing the amount of fresh water consumed. Although metal binding proteins will first be used to prove our sodium binding concept, the opportunities with it are endless.

Safety

Safety is ensured for our project through various means, one of which is being safe in the lab. Workplace Hazardous Materials Information System (WHMIS) training is required by all team members before lab work is performed, to ensure that safety procedures are met by everyone. Our phage candidates are determined to have a narrow host range (Tétart, Reloila, and Krisch, 1996; Pope et al., 2007). This means that they will only be able to utilize those organisms within it, which adds specificity

to our system. This reduces the risk in the lab and for potential users for the phage to affect them. Our system will require ultraviolet treatment at the end of the apparatus to minimize the phage left in the final water. Many

wastewater treatment plants incorporate a UV treatment prior to treated water being made available for consumption (Figure 5).

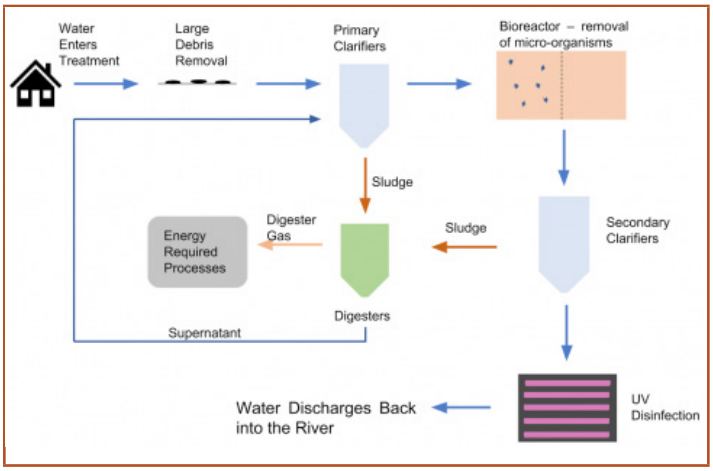

Figure 5. *Wastewater treatment in Lethbridge. General process of how wastewater is treated within the city of Lethbridge.*

We have also considered what would happen if our phage were to escape containment within a wastewater treatment facility. We believe that our engineered phage would not cause disruption to the natural ecosystem. It is likely that if our engineered phage were released into the wild, then natural selection would select against propagating our engineered phage. We can perform further evolutionary analyses on this to predict how long our phage would remain in seawater. Additionally, we can also test phages that have been engineered to contain the essential replication components while removing any virulence factors to avoid any unintended viral infection.

Discussion

An effective desalination process will provide clean drinking water, a basic need that must be met. Current methods of desalination use thermal energy, pressure, or electricity (Figure 5), resulting in slow, costly or inefficient methods (Chung, 2007). Our proposed system of sodium flocculation using synthetic biology demonstrates an effective model for desalination in comparison, as it does not require any of the above methods. Before assembling the complete phage, we will model our fusion protein with the capsid proteins from T4. This will be

useful in identifying whether the ELP and metal-binding protein fusion will bind to the accessory protein HOC, while still functioning properly.

There are multiple ways to test the effectiveness of our system in terms of salinity of the water, brine precipitation, and phage production. To determine if the salinity in the water has dropped below 500 ppm, we will use an osmometer. Additionally, to demonstrate ELP precipitation, a visual assay will be used at the transition temperature. Phage production by our host bacteria *E. coli* will be compared by using a plate with the *E. coli* and phage attached in comparison to our phage-producing *E. coli*.

Negative potential results in terms of binding include the sodium-binding protein failing to bind to the phage, the inability for both the ELPs and the metal-binding proteins to bind together, and the requirement of large amounts of time in order for proper flocculation. It is also possible that some phages will escape into the ocean and harm other organisms, though we have taken into consideration ways to avoid this. Additionally, depending on the orientation of the construct, the introduction of point mutations may introduce premature stop codons, halting translation downstream of the capsid protein.

To model the change in salt concentration, we intend to create a system of differential equations. Based on this model, we will make predictions about the volume, number of cisterns, amount of phage, and concentration of host necessary to reduce the concentration of salt in a sample of seawater to a useful level. From the initial results in our model system (*E. coli* and T4), we will refine our model to better represent the state of the system. Once the model is developed, we plan to extend it to other capsid proteins, phages, and host species to determine the most efficient and cost-effective method of desalination.

Acknowledgements

The Lethbridge High School iGEM team acknowledges the support of the Geekstarter / Mindfuel Program, the University of Lethbridge Department of Chemistry and Biochemistry, Dr. HJ Wieden, Dr. Brian Dempsey, and Dr. Laura Keffer-Wilkes, as well as Chris Isaac, Erin Kelly, Kristi Turton, and Sydnee Calhoun.

References

Adamo GM, Lotti M, Tamas MJ, and Brocca S. Amplification of the CUP1 gene is associated with evolution of copper tolerance in Saccharomyces cerevisiae. Microbiology. 2012; 158: 2325–2335.

Bazan J, Calkosinski I, and Gamain A. Phage display- A

powerful technique for immunotherapy. 1. Introduction and potential of therapeutic applications. Hum. Vaccin. Immunother. 2012; 8:1817-1828.

Chung Y. Introduction to materials science and engineering. CRC Press. 2007.

Government of Canada. Oil Sands: Water Management. Government of Canada. 2015 May.

Government of Canada. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document—Sodium; Health Canada: Ottawa, ON, Canada, 1992.

Guest Contributor. 8 major cities running out of water. EcoWatch. 2015 July.

Herlemann DP, Labrenz M, Jurgens K, Bertilsson S, Waniek JJ, and Andersson AF. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic sea. ISME J. 2011; 5:1571-1579.

Meyer DE and Chilkoti A. Quantification of the effects of chain length and concentration on the thermal behaviour of Elastin-like polypeptides. Biomacromolecules. 2004; 5:846-851.

National Geographic. Why Cape Town is running out of water, and who's next. National Geographic. 2018 March.

OECD: Estimate of support to agriculture. 2012.

Pope WH, Weigele PR, Chang J, Pedulla ML, Ford ME, Houtz JM, Jiang W, Chiu W, Hatfull GF, Hendrix RW, and King J. Genome Sequence, Structural Proteins, and Capsid Organization of the Cyanophage Syn5: A "Horned" Bacteriophage of Marine Synechococcus. J Mol Biol. 2007; 368(4): 966-981.

Raytcheva DA, Haase-Pettingell C, Piret J, and King JA. Two novel proteins of Cyanophage Syn5 compose its unusual horn structure. J virol. 2014; 4:2047-2055.

Ren ZJ and Black LW. Phage T4 SOC and HOC display of biologically active, full-length proteins on the viral capsid. Gene. 1998; 215(2):439-444.

Rosano GL and Ceccarelli EA. Recombinant protein expression in *Escherichia coli*: advances and challenges. Front Microbiol. 2014; 5:172.

SJTU-BioX- Shanghai iGEM team. Cyano Pacman. 2015.

Stetefeld J, Jenny M, Schulthess T, Landwehr R, Engel J, and Kammerer RA. Crystal structure of a naturally occurring parallel right-handed coiled coil tetramer. Nat Struct Biol. 2000; 7:772-776.

Suttle CA and Chan A. Marine cyanophages infecting oceanic and coastal strains of Synechococcus: abundance, morphology, cross-infectivity and growth characteristics. Mar Ecol Prog Ser. 1993; 92:99-109.

Tétart F, Reloila F, and Krisch HM. Bacteriophage T4 host range is expanded by duplications of a small domain of the tail fiber adhesion. J Mol Biol. 1996; 258(5):726-731.

Techradar. Our smartphone addiction is costing the Earth. 2015.

University College London iGEM team. Welcome to plastic republic. 2012.

Wang P, Wesdemiotis C, Kapota C, and Ohanessian G. The Sodium Ion Affinities of Simple Di-, Tri-, and Tetrapeptides. J Am Soc Mass Spectrom. 2007; 18: 541-552.

Weinbauer M, Bonilla-Findji O, Chan AM, Dolan JR, Short SMMM, Simek K, Wilhelm SW, and Suttle CA. Synechococcus growth in the ocean may depend on the lysis of heterotrophic bacteria. J Plank Res. 2011; 33:1465-147.

World of Earth Science. Desalination. Encyclopedia.com. 2003.

Yap ML and Rossmann MG. Structure and function of bacteriophage T4. Future Microbiol. n.d.; 9:1319-1327.

Zhao Y, Temperton B, Thrash JC, Schwalbach MS, Vergin KL, Landry ZC, Ellisman M, Deerinck T, Sullivan MB, and Giovannoni SJ. Abundant SARII viruses in the ocean. Nature. 2013; 24:357-494.

Zimmerman M, Udagedara SR, Sze CM, Ryan TM, Howlett GJ, Xiao Z, and Wedd AG. PcoE-A metal sponge expressed to the periplasm of copper resistance Escherichia coli. IMplication of its function role in copper resistance. J inorg Biochem. 2012; 115:186-197.