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Design and analysis of of synthetic MMP-9 sensors as diagnostic tools for concussions

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Abstract

While millions of individuals suffer concussions caused by head trauma each year, an accurate, reliable, and portable diagnostic for concussions does not yet exist, making a novel concussion detection system necessary. Matrix Metalloproteinase-9 (MMP-9) is a biomarker whose concentration in the blood increases after suffering a head injury. We designed a chimeric protein sensitive to MMP-9 and planned to compare it to a MMP-9 repressor system to detect the status of an individual's condition from a blood sample. When MMP-9 is tested, the chimeric protein system will use β-lactamase to hydrolyze nitrocefin producing a brown color and the repressor system will produce GFP because MMP-9 cleaves off the repressor from pPROT allowing transcription to occur. The results of the MMP-9 sensitive chimeric repressor system showed that by adding MMP-9 there was an increase in GFP production.

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- The 2015 Pittsburg iGEM team for kindly gifting us their chimeric repressor system plasmids. Without their gift, our project would not have been possible.

Introduction

What is a Concussion?

Each year, approximately 1.6-3.8 million concussions occur in sports and recreational activities (Daneshvar et al. 2011) and many concussions are still not reported. A concussion is a "a complex pathophysiological process affecting the brain, induced by biomechanical forces" which can be caused by either direct or indirect impact to the head, face, neck or elsewhere leading to "rotational acceleration of the brain" (Tator 2013). After receiving a concussion there are multiple symptoms that can occur such as loss of consciousness or amnesia, behavioral changes, and cognitive and somatic symptoms. Many times these symptoms can be underreported by the person in question or it is difficult to gage how severe the symptoms are such as the severity of headaches and slowed reaction times (Daneshvar et al. 2011).

Once a concussion has been received it is much easier to sustain another, and this phenomenon is called the second-impact syndrome (Tator 2013). Repeated concussions can cause chronic traumatic encephalopathy as well as negative long-term effects on the brain. These repeated injuries could lead to posttraumatic brain degeneration, which may cause dementia and movement disorders later in life (Tator 2013). Due to the probability of neurological damage caused by concussions, it is important to have accurate testing and management tools to diagnose concussions and keep those who suffer from concussions safe.

There are many types of concussions tests, which can be broken up into two large groups: assessment tools and management tools. Within these two groups there are physical examination tools, symptom checklists, sideline assessment tools, balance assessment tools, computerized neurocognitive testing and neuroimaging. Despite these tools, no test has been shown to quickly and reliably assess concussions in all cases. Further, they are costly and must be performed in a hospital or by trained specialists.

Biomarkers

The term "biomarker" refers to a broad category of medical signs that indicate the medical status of a patient, so these biomarkers can then be used as surrogate endpoints. In order for a molecule to be regarded as a biomarker for a disease, the molecule has to be able to provide accurate information to researchers and physicians to the presence of a disease (Strimbu 2010). An example of such a biomarker would be hemoglobin ions, which increase after an acute

ischemic stroke. This increase can be used to detect acute ischemic strokes at a sensitivity level of 70.2% (Huang et. al. 2009)

A study completed in 2015 revealed that the levels of several enzymes were increased in blood within hours of a concussion. One such enzyme is Matrix Metalloproteinase 9 (MMP-9), which was elevated 3.6-4.5 fold up to eight hours after head injuries (Rongzi et al. 2014). This finding raises the possibility that MMP-9 could be used as a biomarker for concussions.

Matrix Metalloproteinase-9

MMP-9 comes from a family of multi-domain proteins called Matrix Metalloproteinase (MMPs). MMPs generally function in the extracellular environment of cells by degrading both matrix and non-matrix proteins. These proteins play central roles in morphogenesis, wound healing, and in progression of diseases such as cancer and chronic tissue ulcers (Nagase et. al. 2006).

MMP-9 plays a vital role in the promotion of tumor growth during the early stages of tumorigenesis. MMP-9 is also known to regulate different pathological remodeling processes that involve inflammation. MMP-9 does this by degrading extracellular matrix proteins while activating cytokines and chemokines to regulate tissue remodeling (Yabluchanskiy et al. 2013). MMP-9 contains three fibronectin type II repeats with a high affinity for collagen. This draws the MMP-9 enzyme to the collagen, enhancing the rate of hydrolysis. MMP-9 cleaves many other proteins, and three different families of substances have been identified. The largest protein target family contains a Pro-X-X-hy-(Ser/Thr) motif where X is any residue and hy is a hydrophobic residue. The second group is defined as Gly-Leu-(Lys/Arg) motif, and the third family had all peptides cleaved after an Arg residue (Kridel et al. 2001). This emphasizes the vast diversity of protein targets cleaved by MMP-9 and the specificity of MMP-9's cleavage sites.

Chimeric Proteins

Chimeric proteins are a single protein product generated from the fusion of proteins or protein domains from several different proteins. Chimeric proteins can be created using a PCR procedure to combine multiple gene fragments. Some uses of chimeric proteins are to study the folding of a protein or to evaluate its stability (Tripathi et. al. 1999). Chimeric proteins have also been used in medical applications such as creating a fusion protein to increase the immune response to tumor cells in people with breast cancer (Grandori et. al. 1997).

Project Goal

Currently there are limited tests for accurately diagnosing concussions. MMP-9 is a demonstrated biomarker for concussions and could be an important factor in the development of new concussion diagnostics. Therefore, the goal of this project is to create an assay for detecting MMP-9 in blood samples, which can be used to evaluate whether someone is suffering from a concussion. We will build and test two different models for detecting MMP-9 activity.

One method is to create a chimeric biosensor similar to the one created by Nirantar et al. This chimeric protein will be comprised of β-lactamase attached to its inhibitor, BLIP, by a linker containing the MMP-9 cleavage site (Nirantar et al. 2013). When MMP-9 cleaves this linker, BLIP will be separated from β-lactamase. β-lactamase can then hydrolyze nitrocefin resulting in a brown color. This color change will indicate an increase in MMP-9 in the blood and could therefore be used to evaluate if someone suffered a head injury (Figure 1).

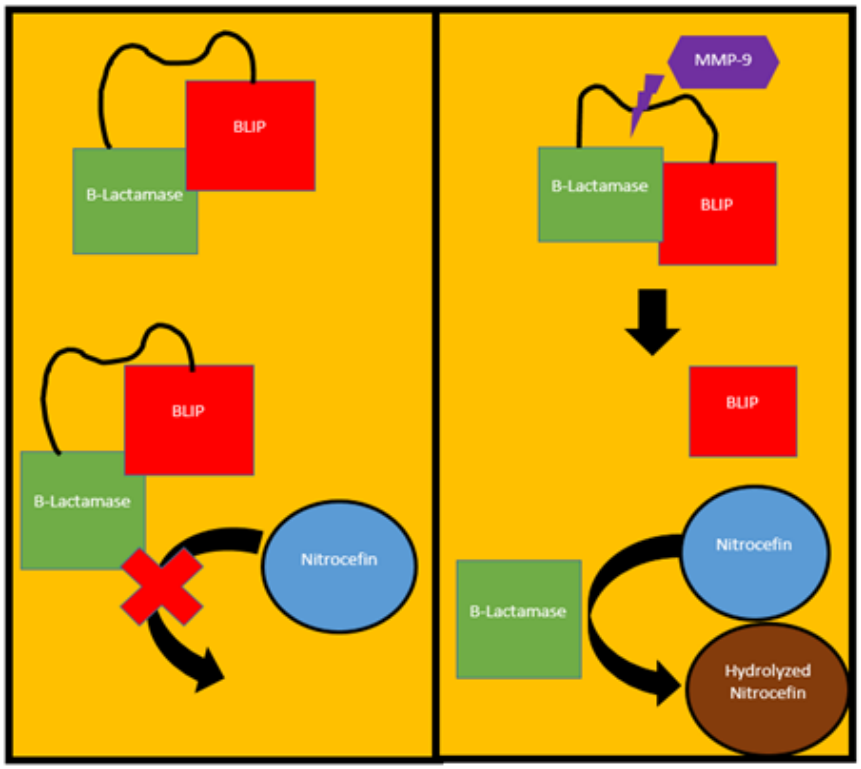


Figure 1: β-lactamase and BLIP held together by a protein linker containing a cleavage site for MMP-9 without (left side) and with (right side) MMP-9 present. When MMP-9 separates BLIP from β-Lactamase, nitrocefin with be hydrolyzed by the β-Lactamase, resulting in a brown color change.

To understand how our chimeric protein system compares to other methods of detecting MMP-9, we will analyze the function of a previously designed MMP-9 sensor that utilizes an MMP-9 sensitive repressor to repress the expression of the reporter gene GFP (Pitt iGEM 2015, Figure 2). The chimeric repressor protein binds the pPROT promoter and inhibits the expression of GFP. The chimeric repressor contains an MMP-9 cleavage site, and will dissociate from the pPROT promoter upon MMP-9 cleavage. The removal of this inhibitor will allow for *E. Coli* RNA polymerase to bind to the pPROT resulting in the production of the GFP.

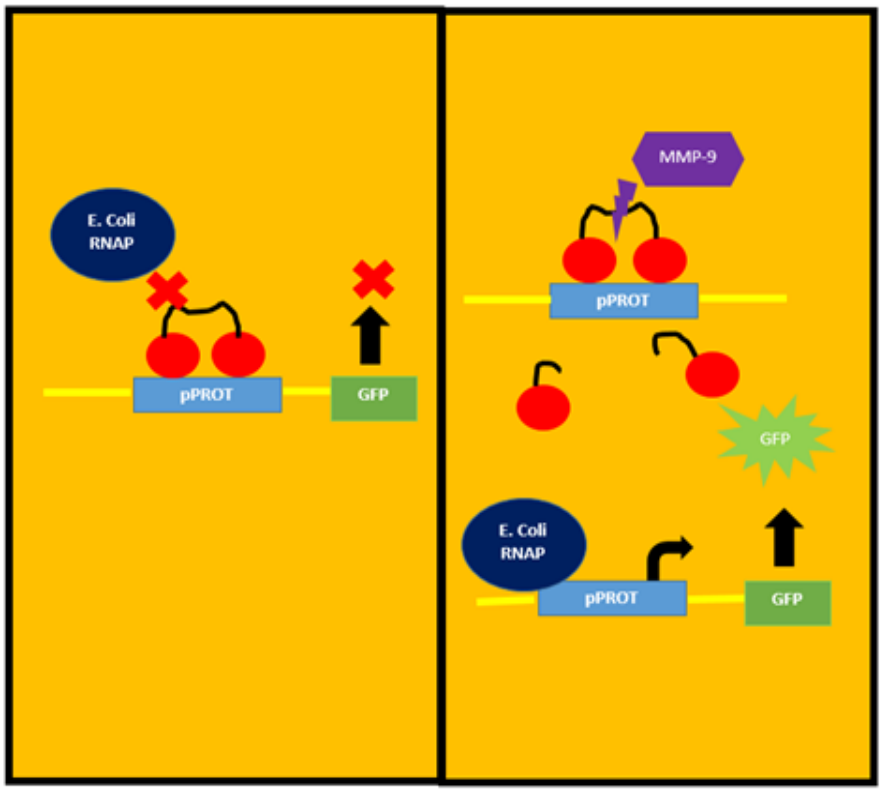


Figure 2: pPROT is bound by a chimeric repressor that contains a cleavage site for MMP-9 and represses GFP expression in the absence of MMP-9 (left side). When MMP-9 is present, it cleaves the repressor and activates transcription from pPROT, and GFP is produced (right side).

Methods

Designing MMP-9 Sensitive Systems

In order to design the chimeric protein system, A Plasmid Editor (ApE) was used to map out the specific DNA sequence. The MMP-9 sensitive chimeric protein that was designed contains β-lactamase, BLIP-Linker and pSB1C3, a plasmid backbone containing a gene that codes for chloramphenicol (CAM) resistance (Figure 3).

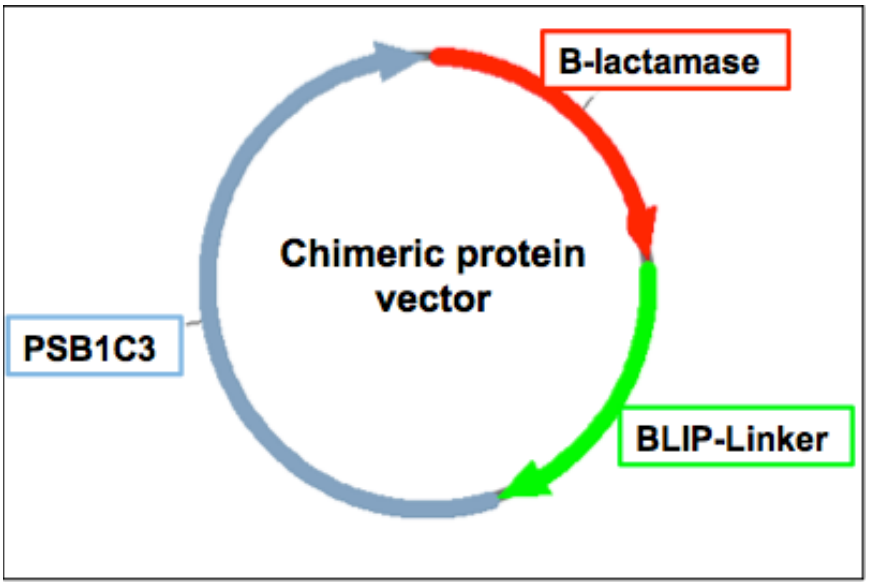


Figure 3: Designed MMP-9 sensitive protein vector containing β-lactamase, BLIP-linker and PSB1C3, a gene that codes for chloramphenicol resistance

The MMP-9 sensitive chimeric repressor system contains two vectors: one is the pPROT and GFP (Figure 4a) and the other is the pPROT repressor (Figure 4b). The plasmids were obtained from the 2015 Pitt iGEM team.

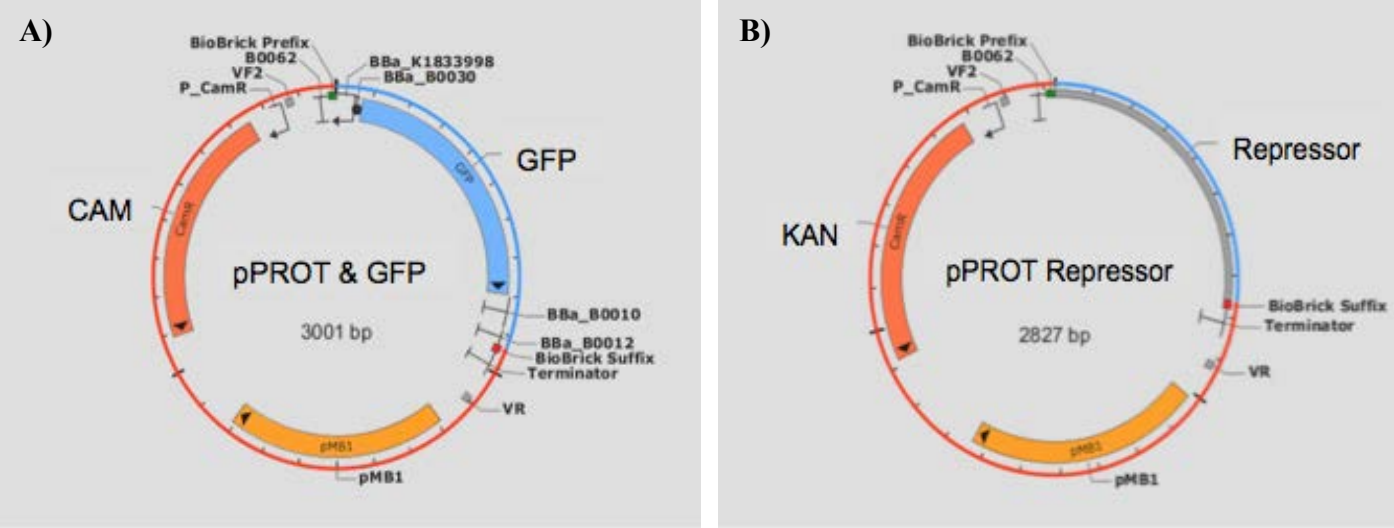


Figure 4: A) The pPROT promoter and the GFP gene along with chloramphenicol (CAM) resistance. B) The pPROT repressor and kanamycin (KAN) resistance

Construction of β-lactamase

In order to construct the β-lactamase sequence, A Plasmid Editor (ApE) was used to map out the specific DNA sequence. The DNA sequence was derived from the experiment ran by Nirantar et. al. (Nirantar et. al. 2013). The sequence was taken from the pSB1A3 template obtained from the Registry of Standard Biological Parts (parts.igem.org). The β-lactamase sequence can be found in Appendix 1. The 3' and 5' primers for B-lactamase were both obtained from the Registry of Standard Biological Parts. The sequence of these primers can be found in Table 1. DNA sequencing and insert amplification was performed using the primers VF2 and VR (Table 1) as necessary.

Table 1: List of primers and the sequences used in this study

Primer Name	Primer Sequence
3' β-lactamase primer sequence	ACGCTGCAGCGGCCGCTACTAGTGTAATGCTTAATCAG
5' β-lactamase primer sequence	TCCTTCGAGAGAAAAAGGAAGAGTATGA
BLIP-linker prefix sequence	GTTTCTTCGAATTCGCGGCCGCTTCTAG
BLIP-linker suffix sequence	TAACTGCAGCGGCCGCTACTAGTA
VF2 primer sequence	TGCCACCTGACGTCTAAGAA
VR primer sequence	ATTACCGCCTTTGAGTGAGC

Construction of BLIP-linker

The BLIP-linker sequence was created similarly to the β-lactamase sequence both using ApE and deriving the sequence from the experiment by Nirantar et. al. (2013). The BLIP-linker sequence was synthesized by Integrated DNA Technologies (Coralville, Iowa) and the DNA sequence can be seen in Appendix 2. The prefix and suffix sequences for BLIP-linker were also obtained from the Registry of Standard Biological Parts. The sequences for these primers can be found in Table 1.

Plasmid

The plasmid backbone, pSB1C3, was ordered from the Registry of Standard Biological Parts and contained a strong constitutive promoter, J23119. Both the sequence for the pSB1C3 backbone and the J23119 promoter can be found in Appendix 3.

Cloning of β-lactamase and BLIP-linker into Biobrick plasmid

A PCR reaction was setup to create the BLIP-linker and β-lactamase. The β-lactamase reaction was set up using the 5' and 3' β-lactamase primers (10 µM), the pSB1A3 β-lactamase template (0.025 µM), PCR mastermix (New England Biolabs, 25µL), and water (21µL). The BLIP-linker reaction was set up using the 5' and 3' BioBrick primers (10 µM), the synthetic BLIP-linker template (0.025 µM), PCR mastermix (25µL), and water (21µL). The PCR protocol can be found in Table 2. After the PCR reaction was complete, the inserts were purified using the PCR cleanup protocol (Macherey-Nagel). PCR products were digested with Xba1 and Pst1 in preparation for ligation, and confirmed by agarose gel electrophoresis.

Table 2: PCR protocol to cut β-lactamase and BLIP-linker

Temperature	Time	Number of Cycles
95℃	1 minute	1
95℃	30 seconds	30
50℃	45 seconds	30
72℃	1 minute	30
72℃	2 minutes	1

Miniprep of plasmids was prepared using the Macherey-Nagel NucleoSpin Plasmid Miniprep Kit according to the manufacturer's instructions. The pSB1C3 plasmid miniprep (50µL of 0.025µM) was prepared for ligation by digestion with Spe1 (1µL) and Pst1 (1µL) (New England BioLabs), and incubated in a 37℃water bath overnight. The next day the digest was incubated at 80℃for 10 minutes. 10µL of the cut plasmid, along with the undigested plasmid, underwent agarose gel electrophoresis to ensure that the restriction enzymes cut the plasmid properly. The β-lactamase insert miniprep (50µL) was then digested using Xba1 (1µL) and Pst1 (1µL), and the digest was incubated in a 37℃water bath overnight. A reaction cleanup (Macherey-Nagel) was completed and eluted in 30µl of water.

The digest was then ligated and four water/insert combinations were tested. Each reaction had 2µL10x ligation buffer, 2µL purified pSB1C3 plasmid, and 1µL T4 DNA ligase. Different combinations of water and β-lactamase insert were used. The ligation reaction was incubated at room temperature for at least 1 hour.

10µL of each ligation was transformed into *E. coli* strain DH5α cells. The cells with the ligation reaction were incubated on ice for 30 minutes, heat shocked at 42℃ for 1 minute, and then on ice for 5 minutes. 200µL of LB media was added to each transformation and incubated at 37℃ for 2 hours in a shaker. After 2 hours, 200µL of each transformation was plated onto LB Agar + CAM (33 µg/mL) petri plates and incubated overnight at 37℃.

10µL of water was added to 11 PCR tubes and a single colony was resuspended in each. The colonies were re-streaked on a new LB Agar + CAM plates. Then in a new PCR strip, PCR mastermix (12.5µL), 10µM primer VF2 (1.25µL), 10µM primer VR (1.25µL), and the 10µL of water/ bacteria mix was mixed together. The sequences for the VF2 and VR primers can be found in Table 1. A PCR reaction was runfor these 11 tubes following the PCR protocol found in Table 3. Each of the reactions was confirmed by agarose gel electrophoresis for the presence of the β-lactamase insert.

Table 3: PCR colony protocol to check for the β-lactamase insert

Temperature	Time	Number of Cycles
95℃	2 minute	1
95℃	30 seconds	33
55℃	45 seconds	33
72℃	1 minute	33
72℃	2 minutes	1

Transformation of pPROT and MMP-9 repressor

The pPROT-GFP reporter and MMP-9 repressor were generously gifted to us by the Pitt 2015 iGEM team, and transformed into competent cells following the iGEM transformation protocol with a few minor changes (Transformation Protocol n.d.). These changes were that step 4 was not performed and in step 10, only 200µL of each transformation were plated on chloramphenicol (cam) plates. The following day single colonies from the pPROT-GFP plate were picked and suspended in 5mL of LB media that grew overnight and minipreps were completed.

The repressor vector was moved into a kanamycin (KAN) resistant pSB1K3.m1 plasmid backbone. Both the original repressor vector (CAM^R) and KAN vector were each digested with Ecor1 (1µL) and Pst1 (1µL), and incubated overnight in a 37℃water bath overnight. A PCR cleanup (Macherey-Nagel) was completed for the KAN vector and the vector was eluted in 30µl of water. 2µL of Shrimp Alkaline Phosphatase (SAP) was added to the repressor vector , incubated at 37℃for 20 minutes and then incubated at 80℃for 10 minutes. The digests were then ligated and four water/repressor combinations were tested. Each reaction had 2µL10x ligation buffer, 2µL purified KAN plasmid, and 1µL T4 DNA ligase. The ligations were transformed into competent cells and plated onto KAN plates. The following day, single colonies were picked and suspended in 5mL of LB media that grew overnight and minipreps were completed.

The repressor and pPROT-GFP minipreps were co-transformed into competent cells. Six colonies were picked for overnight cultures to be miniprepped the following day. A mini digest was completed in order to check for the pPROT and the MMP-9 repressor. All six colonies contained the correct bands sizes of 889 base pairs for pPROT and 757 base pairs for the repressor. As controls two other transformations were completed with the pPROT and the J23119 empty vector and the repressor and the pSB1K3.m1 vector.

Chimeric Repressor Assay

Sensor extract was prepared according to the protocol created by the Pittsburgh iGEM 2015 team (iGEM 2015) with the following changes: SE RB buffer was used in place of SE WB buffer; the cells were never stored in liquid nitrogen or kept frozen at -80℃but were used immediately after harvest; the cells were lysed using 30 strokes in a dounce homogenizer with

Teflon pestle instead of sonication; lysates were not dialyzed; SE PB buffer was not used, and instead assays below were performed in SE RB buffer.

To assess the effectiveness of the sensor extract in detecting MMP-9, an assay was designed that involved monitoring the fluorescence of each culture with or without the presence of MMP-9. This assay was performed by pipetting eight samples of cell lysates, 200µL per sample, into wells of an opaque white 96-well plate: one pair with only the pPROT vector, one pair with only the repressor vector and two pairs with both vectors. 1µL of 0.2µM of recombinant human MMP-9 (SINO Biologicals) was added to one 200µL sample of lysate from each pair and the lysates were incubated at 32℃for 90 minutes. Samples were then transferred to a 96 well plate and a plate reader (Perkin-Elmer LS 55 Luminescence Spectrometer) was used to assess the fluorescence values of each sample at an excitation wavelength of 485nm and emission wavelength of 515nm.

Microscopy

Cultures containing the pPROT-GFP reporter and/or repressor were viewed on a Zeiss AXIO observer A1 fluorescence microscope. Images of the cells were taken using New Window Idea 5Mp Color Mosaic SPOT supported camera and processed using SPOT Basic software (Diagnostic Instruments, Inc.).

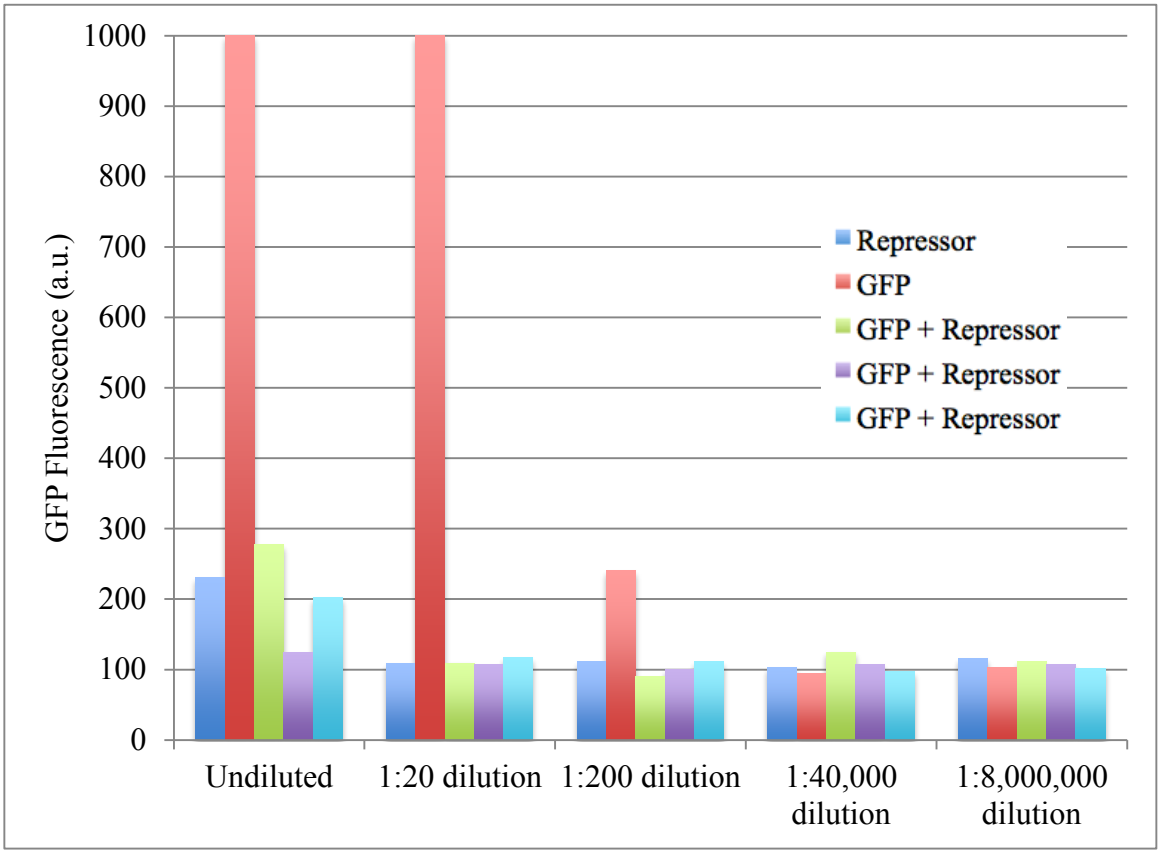
Results

The overall goals of this project were to design a chimeric protein MMP-9 sensitive detection system and compare it to an existing chimeric repressor MMP-9 detection system. Multiple attempts were made to clone the chimeric β–lactamase-BLIP protein (Figure 3) and transform it into DH5α competent cells. Some of these attempts included cloning each individual part of the vector, β-lactamase and BLIP-linker, into different vectors and either cloning the β-lactamase sequence into the vector first or cloning the BLIP-linker sequence into the vector first. Unfortunately, due to time constraints and technical issues, the chimeric protein assay has yet to be created. Therefore, we shifted our focus to the characterization of the chimeric repressor system.

We began by determining the effectiveness of the pPROT promoter and repressor system. Five cultures were grown overnight: repressor only, pPROT-GFP only, and three colonies of repressor plus pPROT-GFP. The day of the testing, the optical density (OD) was taken in order to dilute each culture to the same number of cells. The fluorescence values for each of the cultures at varying dilutions can be seen in Figure 5a. In the undiluted sample, the fluorescence of the culture expressing pPROT-GFP without the repressor was outside the machine's dynamic range of 0-1000, but the other samples all had similar background levels of fluorescence. At the 1:200 dilution all cultures could be compared. As seen in Figure 5a, the repressor and the three repressor plus pPROT-GFP cultures were all around a fluorescence of 100 arbitrary units (a.u.) while the pPROT-GFP only culture was around 250 a.u. This demonstrates that the co-transformation was successful and the repressor is indeed binding to pPROT and decreasing the expression of GFP as expected.

In addition to measuring fluorescence using a plate reader, a fluorescence microscope was used to take pictures of a sample of each of the cultures (Figure 5b). These images also show that the repressor is limiting the expression of GFP in the pPROT-GFP plus repressor samples. As seen in the images there is a high amount of GFP expression in the pPROT-GFP only sample compared to the repressor culture and repressor and GFP cultures.

A)



B)

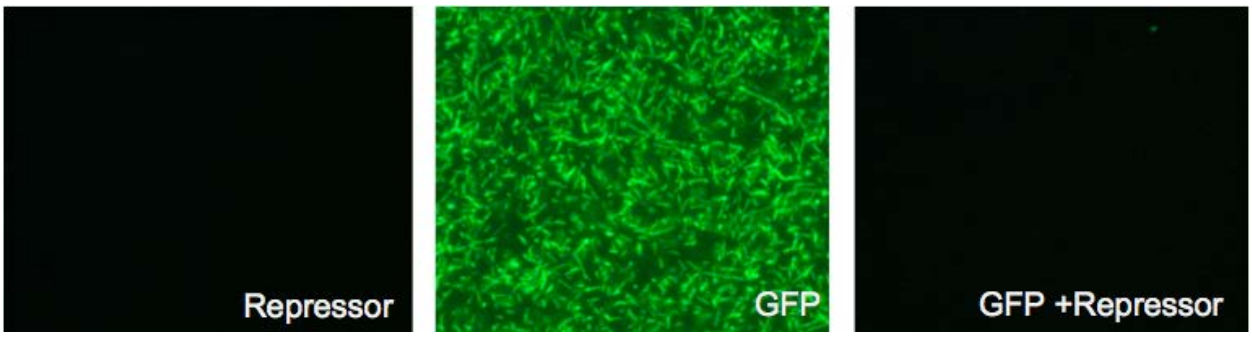


Figure 2: A) GFP fluorescence (a.u.) of Repressor, GFP, and GFP+Repressor cultures at varying dilutions. B) Images of cell cultures under a fluorescent microscope at 400X magnification.

Next, we sought to determine whether the addition of MMP-9 to this system would cleave the repressor protein and result in the expression of GFP. MMP-9 is not membrane-permeable, thus we made lysates from the cell cultures expressing each of the plasmid combinations described previously.

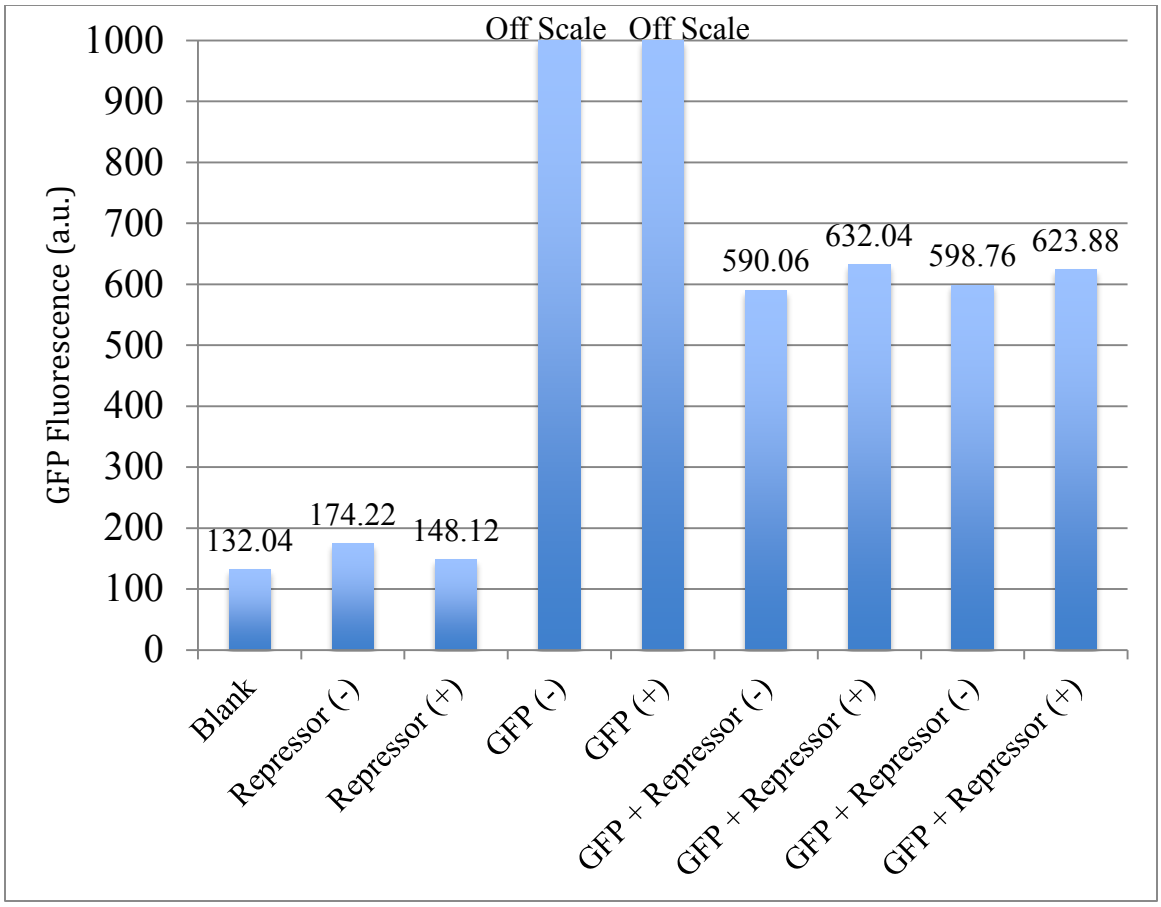


Figure 3: Fluorescence values of cell lysates containing pProt repressor (Repressor), pProt promoter + GFP, or both vectors (GFP + Repressor). All four lysates either received (+) or did not receive (-) MMP-9. A sample of SE RB buffer was used as a negative control (Blank).

As seen in Figure 6, the fluorescence values of the lysates containing only the repressor showed no difference whether or not MMP-9 was added to the lysate. In fact, the fluorescence values were very similar to that of the blank (only SE RB buffer). The fluorescence values of the lysates only containing pPROT-GFP were off scale in both cases as the plate reader used had a dynamic range of 0-1000 a.u. and the amount of fluorescence produced by the GFP-containing lysates were more than 1000 a.u. The pPROT-GFP plus repressor containing lysates produced more fluorescence than the repressor alone, indicating a background level of GFP expression even when the repressor is intact. However, we observed a slight increase of approximately 5-7% in GFP expression in two different lysate samples that contained MMP-9. This outcome suggests that the MMP-9 is cleaving the repressor, causing the repressor to separate away from the pPROT-GFP vector, which results in GFP production in the sample. Due to time constraints, this assay could not be repeated, thus statistical analyses could not be performed.

Discussion

The overall goals of this project were to design a MMP-9 sensitive chimeric protein detection system and compare it to an existing MMP-9 chimeric repressor detection system. It was hypothesized that the MMP-9 sensitive chimeric protein system would be a more robust reported of MMP-9 activity than the MMP-9 sensitive chimeric repressor system. This is because in order to test the effectiveness of the MMP-9 repressor system, it is necessary to produce a cell lysate capable of *in vitro* transcription and translation of the GFP reporter. The chimeric protein detector system would create a protein product which could be more easily activated without in vitro transcription or translation. Further, the chimeric protein could be purified from the bacteria and used to develop the assay into a portable on-site diagnostic.

To achieve our project goals, a chimeric protein vector was designed. Even after multiple attempts at cloning, the MMP-9 sensitive chimeric protein has yet to be properly cloned. We hypothesize that the intermediate cloning products are interfering with the transformation. To avoid this problem, instead of cloning separate pieces together, the final gene product could be created by gene synthesis. This may be a more efficient method of creating this chimeric construct.

As can be seen from the results of the chimeric repressor assay (Figure 6), MMP-9 causes a detectable difference in the GFP levels in cell lysates containing the pPROT promoter and the repressor. Although the change in the overall fluorescence was detectable, it was not as drastic between samples of lysates containing pPROT and repressor and samples of lysates containing pPROT, repressor and MMP-9. Due to time constraints as this assay was only able to be run once so, optimal testing conditions for MMP-9 were not yet determined. Also, no ATP was used during the assay, which could have caused less GFP to be produced. If this assay was optimized, it could be used as an effective tool to detect concussions

Once the assay has been optimized, in order for it to be an effective diagnostic tool the assay needs to be made portable and easy to use. It may be possible to create a concussion detection kit so that, after someone suffers a head injury, their blood sample may be taken. Then either the chimeric protein sensor system or the chimeric repressor sensor system may be used in order to quickly determine if someone has a concussion. In the future this system may be used as a quick, effective diagnostic tool in order to determine a concussion.

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Appendix

Appendix A: β-lactamase sequence from pSB1A3 template

GCCGAATTCGCGGCCGCTTCTAGATGGAAAAAGGAAGAGTATGAGTATTCAACATT
TCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCA
GAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTA
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TTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATT
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AAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTACTAGTAGCGGC
CGCTGCAGGCC

Appendix B: BLIP-linker sequence

GCTGAATTCGCGGCCGCTTCTAGAGGTGGCCCACTGTTCTATAGCGTCGGCAAAATTCCGCGTACACT
GACGGGTGGGCGCCTGCGCCGCCTGGTGACCGCGGCGCTGCTTGCCGCTACCATGGCCGGAAGCACTG
CGGCCGTGGCTCTGGCGGCACCGAAAACCGATCCGGACGAAAATTATTTGACTGACACAAAATATCAG
GCTATCTCATTTGGTATGAGCCGTCAACAAGTGGCGGCGGTTATTGGCACTCGCCCGCATTGTTCCGGT
ACCGGTAGTGACGGCCCGTTGGTTTGTTGGGCGAAAAACCAATTTGCCGATCAAACAGGCTCGTTTAC
CTTTAATGCCGCCGATCAGTTAACCCGCAAAGAGAAGGATTATGCCTTTGCTTATGCATGGTATACTCG
CGATTTACCGATGACCATGACCAAGGCCCAATATGAACAGCAATTCACAGTAGGAGACACCCTTGCAG
AAGTGAACGCCCGTGTCGCTGGTACGGCGTGCACCGATCGCTGGGTGGAATATCCGGGTTACCCATCT
CCATCGGGTTGGAAAACGATGATCCAATGCACCGGAACGGTCTCTGAAGCATACCCGGATATTGAGTT
CCATTTCACGGACGGCGTACTCACGCACAAAACATATTATTCGCGCAATGATCCGCACTGATAACCAG
GCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGA
ACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATACTAGTAGCGG
CCGCTGCAGCGT

Appendix C: pSB1C3 backbone sequence and J23119 promoter sequence

PSB1C3 sequence:
TACTAGTAGCGGCCGCTGCAGTCCGGCAAAAAAGGGCAAGGTGTCACCACCCTGCC
CTTTTTCTTTAAAACCGAAAAGATTACTTCGCGTTATGCAGGCTTCCTCGCTCACTGA
CTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGT
AATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAA
GGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCACAG
GCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAA
ACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCT
CTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAG
CGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGC
TCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCC
GGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCA
GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTT
GAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCT
GCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAA
CCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAA
AAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACG
AAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGA
TCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTT
GGTCTGACAGCTCGAGGCTTGGATTCTCACCAATAAAAAACGCCCGGCGGCAACCG
AGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGG
AGTCCAAGCGAGCTCGATATCAAATTACGCCCCGCCCTGCCACTCATCGCAGTACTG
TTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAAACGGCATGATGAAC
CTGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATATTTGCCCATGGT
GAAAACGGGGGCGAAGAAGTTGTCCATATTGGCCACGTTTAAATCAAAACTGGTGA
AACTCACCCAGGGATTGGCTGAGACGAAAAACATATTCTCAATAAACCCTTTAGGG
AAATAGGCCAGGTTTTCACCGTAACACGCCACATCTTGCGAATATATGTGTAGAAAC
TGCCGGAAATCGTCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGCTCA
TGGAAAACGGTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCGTCTTTC
ATTGCCATACGAAATTCCGGATGAGCATTCATCAGGCGGGCAAGAATGTGAATAAA
GGCCGGATAAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCC
AGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGT
TCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCTCCA
TTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTG
ATCTTATTTCATTATGGTGAAAGTTGGAACCTCTTACGTGCCCGATCAACTCGAGTG
CCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGT
ATCACGAGGCAGAATTTCAGATAAAAAAAATCCTTAGCTTTCGCTAAGGATGATTTC
TGGAATTCGCGGCCGCTTCTAGAG

J23119 sequence:
TTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGC