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TITLE: Peptidic Inhibitors of N-myc for Treatment of Neuroblastoma

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The goal of this project is to develop drug-like peptides that can directly bind to the oncogenic transcription factor N-myc and block its ability to activate downstream genes. N-myc, an oncogenic transcription factor, is an attractive target for the treatment of Neuroblastoma. However, N-myc is thought to be an "undruggable" target as it is involved in protein-protein interactions that are difficult to modulate with small molecules. Here, we attempted to develop drug-like peptides that can directly bind to N-myc and block its activation of downstream genes. In the past year, we have attempted to use mRNA display, an *in vitro* selection technology, to develop short peptides that can bind to N-myc. We originally identified in several sequences resulting from an in vitro selection targeting N-myc via next generation seqeuncing, however, subsequent experiments have determined that the peptides resulting from this selection can unexpectedly bind the matrix used for target immobilization, even in the presence of biotin. Our results also demonstrate the importance of using a hydrophilic immobilization matrix to avoid a strong undesirable hydrophobic bias in the library.

15. SUBJECT TERMS
mRNA display, Neuroblastoma, myc, peptide, SUPR peptide, protein-protein interactions, antibody, *in vitro* selection, directed evolution

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TABLE OF CONTENTS

A. INTRODUCTION

The goal of this project was to develop drug-like peptides that could directly bind to the oncogenic transcription factor N-myc and block its ability to activate downstream genes. N-myc is amplified in up to 40% of Neuroblastoma cases and is a highly attractive cancer target, as it is hypothesized to be a causative factor in Neuroblastoma. Previous attempts at targeting N-myc have been unsuccessful as small molecule therapeutics are generally poor at blocking protein-protein interactions such as those between N-myc and its downstream partners. In this project we attempted to generate drug-like peptides that could bind with antibody-like specificities and affinities to N-myc and that were resistant to proteolytic degradation. In the third and final year of the project, our goals were to 1) repeat mRNA display selections to generate novel peptides targeting N-myc, 2) to increase the affinity of our selected peptides for N-myc, and 3) to test these peptides for their ability to inhibit N-myc function.

B. KEYWORDS

mRNA display, Neuroblastoma, myc, peptide, SUPR peptide, protein-protein interactions, antibody, *in vitro* selection, directed evolution

C. ACCOMPLISHMENTS

1. What were the major goals of the project?

The major goal of the project for Year 3 was to address Specific aim 1: To Engineer Drug-Like Peptides that Bind N-myc by SUPR mRNA Display. Within Aim 1, there were two major goals: 1) *In vitro* selection of SUPR peptides via mRNA display, and 2) to characterize *in vitro* activity of N-myc binding SUPR peptides. Within these major goals, there were also subgoals as shown below:

Specific Aim 1: To Engineer Drug-Like Peptides that Bind N-myc by SUPR mRNA Display	Time-line (MM/YY)	Percent Complete
Major Task 1: In Vitro Selection of SUPR Peptides via mRNA Display		
Subtask 1: Synthesize Unnatural Amino Acid (Aib)/tRNA	11/15	100%
Subtask 2: Synthesize and express N-myc target	11/15	100%
Subtask 3: Synthesize mRNA display libraries. Order synthetic DNA library 1. Order synthetic DNA library 2. PCR, *in vitro* transcribe using T7 RNA Polymerase, Ligate synthetic DNA linker, translate using rabbit reticulocyte lysate with unnatural Aib-tRNA	1/16	100%
Subtask 4: Perform in vitro selection for binding to N-myc target and for stability against protease digestion. Repeat selection until binding of ^{35}S radiolabeled peptides to N-myc target is detected	4/16	0%

Subtask 5: Using the DNA pool from Subtask 4, perform binding competition with c-myc peptide/protein to select against sequences that bind both N- and c-myc.	4/16	0%
Subtask 6: Illumina sequence DNA pools from Subtasks 1.4 and 1.5. Rank order sequences. Perform SAR analysis of sequences.	5/16	0%
Milestone(s) Achieved: *Isolation of SUPR Peptide Sequences that putatively bind N-myc. At least 5-10 top candidates for characterization in Aim 2*	5/16	25%
Specific Aim 2: To Characterize the Properties of N-myc Binding SUPR Peptides		
Major Task 2: Characterize In Vitro Activity of the N-myc Binding SUPR Peptides		
Subtask 1: Chemically synthesize peptides from Aim 1. HPLC purify and use MALDI-TOF to confirm correct mass. Use HPLC to confirm purity.	7/16	0%
Subtask 2: Determine binding constants, kinetic constants using Biacore.	8/16	0%
Subtask 3: Determine secondary structure via circular dichroism	8/16	0%
Subtask 4: Express N-myc-CFP and Max-YFP proteins. Purify via Ni-NTA column. Test to confirm binding of N-myc/Max is seen via FRET. Test effect of SUPR peptides on N-myc/Max binding.	9/16	0%
Subtask 5: Test N-myc/Max DNA binding via gel shift assay. Test effect of SUPR peptides on N-myc/Max binding to DNA.	10/16	0%

2. What was accomplished under these goals?

Initially, it seemed that we were in a good position to complete subtasks 1-4 and beyond and eventual complete Major task 1 during Year 2. However, in Year 2, we encountered a major setback (summarized below) that required us to redo several subtasks. Thus, in Year 3, we had to repeat our initial discovery experiments and as a result, we have been unable to begin work on Major Task 2 since it is dependent on the completion of Major task 1.

Accomplishments for Year 3 are organized by Subtask and described below.

Major Task 1: In Vitro Selection of SUPR Peptides via mRNA Display

<u>Subtask 1: Synthesize Unnatural Amino Acid (Aib)/tRNA</u>

Completed in Year 1

Subtask 2: Synthesize and express N-myc target

Completed in Year 1

Subtask 3: Synthesize mRNA display library

Completed in Year 1

Subtask 4: *In vitro* selection vs. N-myc peptide

Summary of Work in Previous Funding Years:

In Year 1 of this project, we initially identified several families of putative N-myc binding peptides using mRNA display and Illumina sequencing. After testing the binding of these putative peptides by measuring the amount of radioactively-labeled peptides that could be pulled-down on bead immobilized N-myc target, we noted several peptides that showed potentially higher levels of binding to beads with N-myc vs. beads without N-myc (i.e., background binding). However, further analysis suggested that these peptides were likely artifacts due to the magnetic beads that we used for target immobilization.

Our hypothesis is that while these magnetic beds show higher target binding and lower nonspecific binding in a single binding experiment, over the course of several cycles of repeated binding followed by amplification of the sequences that bind to these beads of binding that these specific magnetic beads have a subtle but significant effect on the sequences found in our peptide libraries. Namely, these magnetic beads show a bias for hydrophobic sequences, as evidenced by the fact that many of the peptide sequences that resulted from in vitro selections on these beads are very hydrophobic. By using a fast and crude measure of a peptide or protein's hydrophobicity/hydrophilicity called a GRAVY score (Grand Average of Hydropathy (1)), we demonstrated that the peptide sequences that resulted from our initial selections targeting N-myc were highly hydrophobic, with most peptide scores >1 (negative GRAVY scores indicate hydrophilic while positive GRAVY scores indicate hydrophobic character, and the more extreme a GRAVY score is, the more hydrophobic or hydrophilic a sequence is).

Work in the 3rd Year of the Project:

With the data suggesting that the sequences that we discovered were artifacts of the magnetic beads that we were using, we decided to restart Subtask 4 and repeat the selection to obtain N-myc binding peptides. Our plan was to take two complementary approaches:

1. Repeat the mRNA display selection using a similar peptide library (M$\mathbf{X}_9\mathbf{3}$; where **X** = any one of the 20 natural amino acids and **3** is one of L, M, or V) and N-myc target (Biotin- GGSLQAEEHQLLLEKEKLQARQQQLLKKIEHART-amide), however we would use neutravidin agarose beads to immobilize the target vs. streptavidin magnetic beads that we had used in Years 1 and 2.

2. To express, and purify biotinylated N-myc protein, and use this as a target for mRNA display selection using the same M**X**$_9$**3** library described above. This approach would have the advantage of us being able to target the entire protein, which could increase the likelihood of isolating N-myc binding peptides. This would have to be balanced against the chance of having to do more screening at the end of the project as it could be possible for our peptides to recognize a region of N-myc that would not affect protein function. We would again immobilize the biotinylated N-myc protein on Neutravidin agarose beads to avoid magnetic beads issues as above.

Results from Selection 1:

Using the M**X**$_9$**3** library, we targeted the leucine zipper region of the N-myc protein, which has previously been identified as important for downstream N-myc function. This leucine zipper region dimerizes with other transcription factors, which is necessary for the dimers to bind to DNA and effect the transcription of downstream genes. Thus, our hypothesis was that a peptide that could bind well to the leucine zipper region could then block dimerization, thereby blocking downstream gene activation.

We performed the mRNA display selection using standard protocols, ensuring that we used neutravidin agarose beads for target immobilization. After three rounds of mRNA display selection, we submitted the pool for Illumina sequencing, then analyzed the frequency of sequences in the pool. The top 20 sequences are shown in **Figure 1**.

Our first observation of the top 20 sequences is that they are highly homologous to each other. In Figure 1, we have grouped them into sequences that have an Asp at position 7 (left column) and those that do not (right column), but the sequences in both columns resemble each other at other positions throughout the peptide. A second

	Sequence	ppm	Gravy		Sequence	ppm	Gravy
NM3-1	MWFFLIDTRLL	26932	1.12	NM3-2	MYGFLLTPMML	84753	1.29
	MWYFLLDPRLL	1574	0.64		MFGFLLTPRML	1027	1.10
	MWYFLLDSRWL	1249	0.32		MWGFMLTPAML	976	1.16
	MWFFVLDTRLL	876	1.09	NM3-4	MFGFILPTFML	940	1.77
	MWFFEIDPRKL	1182	-0.21		MWGFFISATML	920	1.36
NM3-3	MWGFILDTRLL	2032	0.85		MIGFLLIPKWL	909	1.49
	MFGFLLDPRWL	1772	0.63		MFGFILPTYWL	821	1.19
	MWGFILDTRWL	1421	0.46		MFGFLLNPLLL	798	1.72
	MWGFLIDARLL	803	1.06		MDLYALPRWYW	973	-0.52
	MWAFFLDSRLL	1355	0.88		MWHFLISPSML	883	0.92

Figure 1. **Putative N-myc-binding sequences identified via Illumina sequencing.** Sequences from the MX$_9$3 library from Round 3 are shown above. The frequency of each peptide sequence in the library is represented in parts per million (ppm). Hydrophobic amino acids (I, L, M, V) are shown in green, hydrophilic amino acids are shown in yellow, aromatic amino acids (F, W, Y) are magenta, positive amino acids (R, K, H) are blue, and negative amino acids (D, E) are red. The C-terminal amino acids encoded by the 3' primer binding site used for PCR are not shown (typically, GSGTSGSS or VVVRLAP). The peptides show significant homology to each other, and have been grouped based on the presence (left column) or the absence (right column) of an Asp appearing at position 7. GRAVY scores for the peptides are also shown, with positive scores representing hydrophobic peptides and negative scores representing hydrophilic peptides.

observation that the GRAVY scores of the peptides are moderate, with many sequences falling between 0-1, indicating that they are somewhat hydrophobic, but not overly hydrophobic where nonspecific binding or poor water solubility is expected. Since protein-protein interactions are energetically driven by hydrophobic burial, some moderate hydrophobicity seems necessary for a peptide to bind to a protein surface. Our analysis of our GRAVY scores seems to indicate that peptides with GRAVY scores <1 seem to make good peptide ligands, and several of the peptides in this project seem to meet this generalization. Third, analysis of the secondary structure of the peptides using online prediction programs seem to indicate that these peptides have high alpha-helical propensity. While these programs generally have limited predictive accuracy, the high alpha-helical prediction is consistent with the model of an N-myc binding peptide binding in a Leucine zipper-type conformation to the leucine zipper region of N-myc. Further, it is tempting to speculate that our putative peptides have a peptide sequence that is consistent with a leucine zipper fingerprint, with leucine amino acids located roughly one full turn (~7 amino acids) away.

We chose four peptides that were representative sequences to further characterize and test for N-myc binding. We chose the most abundant sequences, NM3-1 (**MWFFLIDTRLL**; abundance = 26,932 ppm) and NM3-2 (**MYGFLLTPMML**; abundance = 84,753) for further study since higher abundance often indicates higher functionality (i.e., binding to target). We also chose two other less abundant sequences: NM3-3 (**MWGFILDTRLL**; abundance = 2,032) was selected since it has both a Gly at position three and an Asp at position seven in the sequence, which are found in several peptide sequences (**Figure 1**). NM3-4 (**MFGFILPTFML**; abundance = 940) was selected since it swaps the position of Pro at position seven and Thr at position eight (e.g., compared to the related peptide NM3-2, which contains a Thr at position seven and Pro at position eight).

These four peptides were then tested for binding against the N-Myc target using a radioactive binding assay. We synthesized the DNA coding for these peptides, transcribed the DNA into mRNA, then radiolabeled the peptides as mRNA-peptide fusions via *in vitro* translation reactions in the presence of ^{35}S-Methionine, as previously described (2). After purification, these peptides were incubated with biotinylated N-Myc target on neutravidin agarose beads, the beads were washed, and the amount of radioactivity present in the washes and beads was determined using liquid scintillation counting. **Figure 2** shows the results of this radioactive binding assay, where blue bars represent binding of the peptides to biotinylated N-myc target immobilized on Neutravidin agarose, and as a control, red bars represent binding to biotin-blocked Neutravidin agarose. As shown in **Figure 2**, no difference is observed between the biotin-blocked and N-myc target samples, indicating that the peptides are binding to the neutravidin agarose beads and not specifically to the N-myc target.

This result is unexpected and surprising; previously, neutravidin-binding peptides were isolated by one group (3) using phage display. However, these peptides were competitive with biotin and bound to the biotin-binding site on neutravidin. Surprisingly, the peptides that we have isolated bind to neutravidin even in the presence of biotin, which indicate novel neutravidin-binding peptides. This is also unexpected as we have routinely used neutravidin agarose to immobilize our protein targets and we have not previously isolated neutravidin-binding peptides that can bind even in the presence of

biotin. This result may indicate that the N-myc target is a poor target for recognition by peptides, which makes it more likely for neutravidin-binding peptides to be isolated.

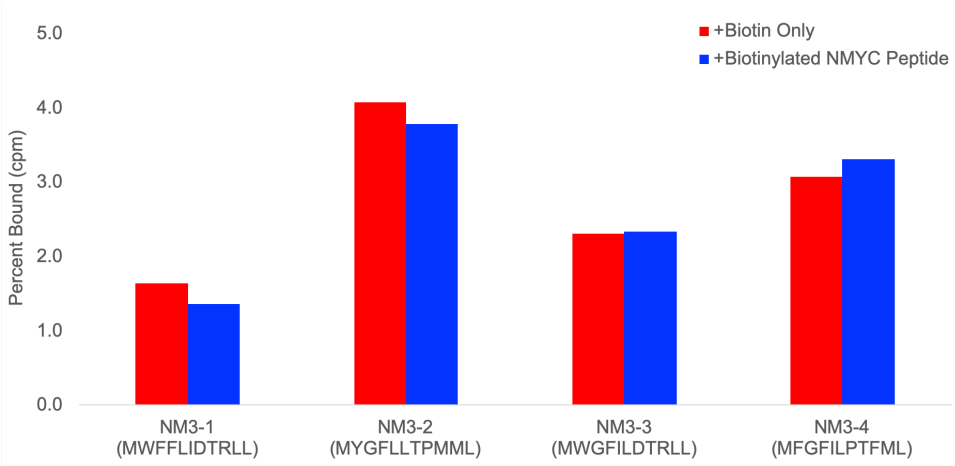

Figure 2. **Radioactive binding assay of putative N-myc binding peptides.** Four peptides from Selection 1 were radiolabeled with ^{35}S-Methionine and tested for binding to biotinylated N-myc target immobilized on Neutravidin agarose beads (blue bars) or to neutravidin agarose blocked with biotin (red bars) as a control. The primary sequence of each peptide is listed under its respective name. Binding is expressed as percent bound, which is the cpm present on the beads divided by the total amount of cpm present in the binding reaction. No significant difference is observed between biotin only and N-myc target samples, indicating that the peptides bind to the neutravidin agarose matrix.

Results from Selection 2:

As an alternate approach, we also attempted to express and purify biotinylated N-myc protein as a target. Our hypothesis was that targeting the entire protein would result in a higher chance of success since the entire protein could present multiple sites where a potential peptide ligand could bind with high affinity. This increased chance of success would have to be balanced with the chance that our peptides would hit a site that would not affect protein function, though better binding sites on a protein generally correlate with protein function.

To do this, our first goal was to clone and express the N-myc protein using an Avi-tag. The Avi-tag is specifically recognized by the BirA enzyme, which specifically biotinylates the Avi-tag peptide (2). This allows a protein expressed in E. Coli to be biotinylated and immobilized for our mRNA display selections. However, upon cloning the gene and then expressing the N-myc-Avitag protein in E. Coli, we observed no protein expression.

Investigating the Effect of Magnetic Beads on Selections

In order to confirm our observation in Year 2 that our choice of magnetic beads as an immobilization matrix had biased our selections, we attempted to experimentally determine the effective bias of the magnetic beads on our peptide library. This experiment was also important to validate our revised strategy of using streptavidin agarose as an alternative immobilization matrix and to test what bias these beads had on the peptide

(A) Pool 0 (Starting Library)
GRAVY <0: 38.5%
GRAVY >0: 61.5%

(B) Pool 1, Streptavidin Agarose
GRAVY <0: 27.4%
GRAVY >0: 72.6%

(C) Pool 2, Streptavidin Agarose
GRAVY <0: 24.8%
GRAVY >0: 75.2%

(D) Pool 1, Streptavidin Magnetic
GRAVY <0: 26.1%
GRAVY >0: 73.9%

(E) Pool 2, Streptavidin Magnetic
GRAVY <0: 1.6%
GRAVY >0: 98.4%

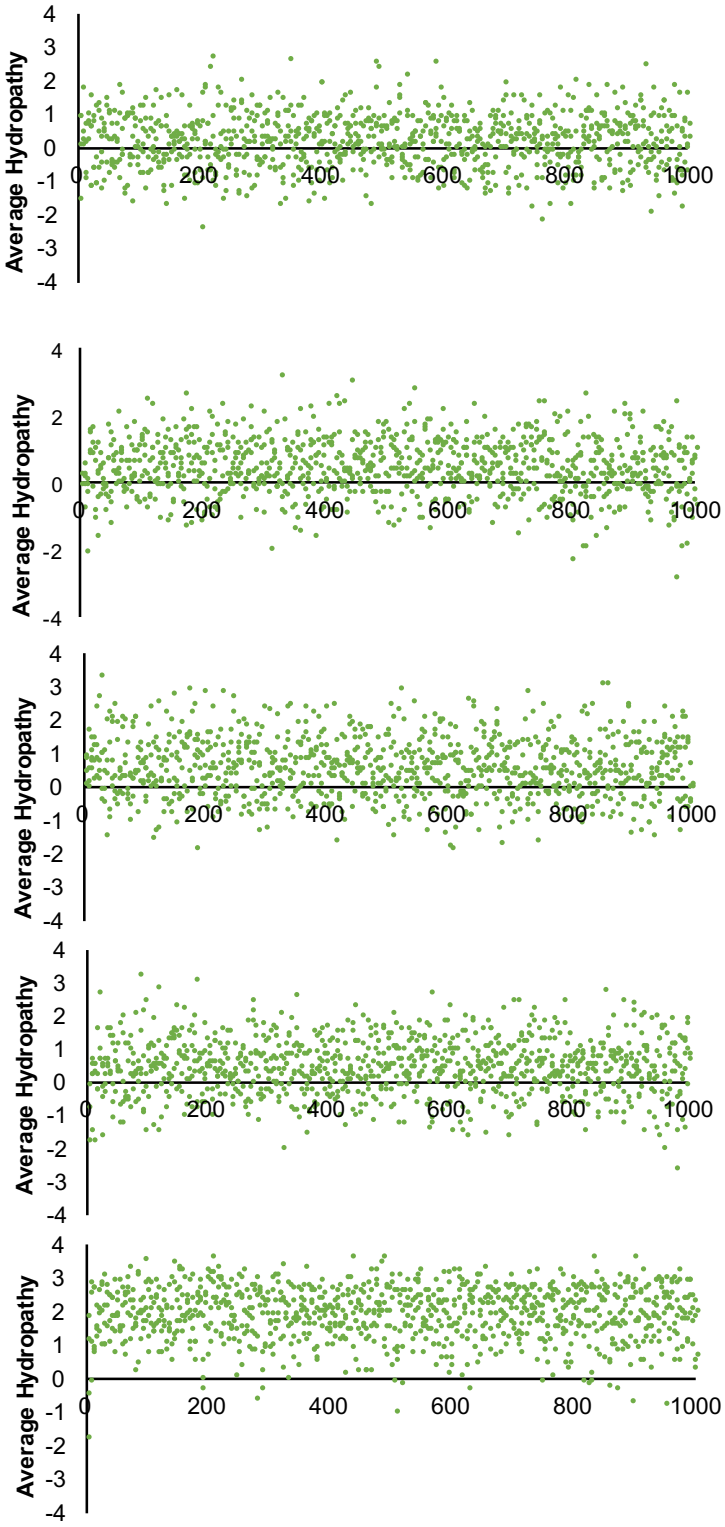

Figure 3. **Effect of Immobilization Matrix on Peptide Pool Hydrophobicity. (A)** The naïve peptide pool used for targeting N-myc (Pool 0) was sequenced using Illumina and the GRAVY scores of the 1,000 most abundant sequences are plotted. This library was used to select against Streptavidin Agarose beads **(B)** and **(C)** or Streptavidin Magnetic beads **(D)** and **(E)**. After two rounds of selection against Agarose beads, a slight bias for more hydrophobic sequences is observed (i.e., more positive GRAVY scores). After two rounds of selection against Magnetic beads, a very strong bias for hydrophobic sequences is observed **(E).**

library. To do this, we performed several selections against either magnetic beads alone (i.e., no target) or streptavidin agarose beads alone. By performing these selections and then Illumina sequencing the resulting pools, we hypothesized that we would be able to see any bias that the choice of matrix had on the composition of the peptide library. As a control, we also sequenced the Pool 0 naïve library that was used as the starting point for all selections.

In the 1,000 most abundant sequences of the pool 0 library (**Figure 2A**), Illumina sequencing shows that ~38% of sequences have hydrophlilic character (GRAVY score < 0) and ~62% have hydrophobic character (GRAVY score >0). This library was then used to select against streptavidin agarose beads (**Figure 2B** and **2C**). After one round of selection (**Figure 2B**), a slight bias toward more hydrophobic sequences is observed (an increase to ~73%), but this bias does not change after a second round of selection (**Figure 2C**; ~75% hydrophobic sequences), which suggests that no significant enrichment of hydrophobic sequences is occurring from Round 1 to Round 2, indicating that binding to agarose beads is not affecting the hydrophobicity of the pool. The increase in hydrophobicity observed from Pool 0 to Round 1 is thus not likely due to bead binding, but rather possibly because of other biases in our mRNA display system, such as translation, transcription, or PCR, etc.

Performing a similar experiment in parallel against streptavidin magnetic beads using the same naïve library, we observed a similar increase in hydrophobic sequences after one round of selection (**Figure 2D;** an increase to ~75%). However, after two rounds of selection against magnetic beads, we observed a significant shift towards hydrophobic sequences (**Figure 2E)** an increase to ~98%). After two round of selection, virtually no hydrophilic peptide sequences are abundant. This indicates that the magnetic beads have a significant effect on the pool composition, resulting in an undeserible shift towards more hydrophobic sequences that are not likely to be soluble as free isolated peptides.

Taken together, these data support the hypothesis that the highly hydrophobic sequences that we observed in Year 2 were due to our inadverdent choice of magnetic beads as our immobilization matrix for our mRNA display selections. It is striking that after only two rounds of selection, nearly all the most abundant sequences in the library are highly hydrophobic, which would introduce significant undesireable bias in our peptide selections. Our data here also suggest that this effect is not as pronounced using streptavidin agarose beads, which supports our strategy of using these agarose beads as our immobilization matrix for reselection against N-myc.

<u>All other subtasks, and Major Task 2</u>

Due to the fact that all other subtasks are dependent upon the successful isolation of N-myc-binding peptides, we were unable to begin work on these subtasks.

3. What opportunities for training and professional development has the project provided?

In the summer of 2018, this project provided an opportunity for training for two high school students – Ms. Skyler Brown (Marymount High School) and Ms. Chloe Kim (Mira Coast High School) through the USC Summer High School Intensive in Next-Generation Engineering (SHINE) Program. The goal of the SHINE program is to give high school

students the opportunity to learn more about and experience research in a University research lab.

Ms. Brown and Ms. Kim were supervised by Dr. Kaori Noridomi, a Postdoctoral Researcher in the Roberts lab. During their time in the SHINE program, Ms. Brown and Ms. Kim learned basic laboratory skills, such as making buffers, how to use pipettes, and use basic laboratory equipment (e.g., UV spectrometers, gel imagers, centrifuges, gel electrophoresis equipment, PCR machines, etc.). They also learned how to perform mRNA display selections via the completion of a homemade training course in mRNA display. They then performed mRNA display selections against the magnetic and agarose beads without any target as described above.

This project thus provided the opportunity for these high school students and aspiring scientists to learn and directly participate in and learn more about research at the University level.

4. How were the results disseminated to communities of interest?

In June, 2018, the PI (Takahashi) spoke to Kindergarten and 4th grade classes at Odyssey Charter School in Pasadena about his research. He was also a speaker in the USC Careers in STEM workshop series on July 24, 2018. Middle and High school students from the local neighborhood surrounding USC were invited to these workshops in order to increase the number of educationally disadvantaged and underrepresented K-12 students who attend college and graduate with STEM degrees.

5. What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report.

D. IMPACT:

1. What was the impact on the development of the principal discipline(s) of the project?

In this project period, we attempted to isolate short proteins (called peptides) against a protein called N-myc. N-myc belongs to a class of protein called “transcription factors” and can turn many genes on, a process that is though to contribute to the development of Neuroblastoma, a type of childhood cancer. N-myc is thought to belong to a category of proteins that are “undruggable,” since many attempts to make drugs that target these proteins have been unsuccessful. Here, we have targeted a domain of N-myc that helps to turn these cancer-causing genes on and obtained a list of potential N-myc binding peptides.

However, these peptides have been shown to bind to neutravidin agarose even in the presence of biotin, which is a highly unexpected result based on our previous experiments. Secondly, during the course of the project, we discovered that our choice of immobilizing the N-myc target on magnetic beads for mRNA display selection experiments was extremely suboptimal, as the magnetic beads have a significant,

unintended and undesirable bias resulting in enrichment of highly hydrophobic sequences that are unlikely to be useful because of their poor water solubility.

Although the project did not meet its intended goals, the impact on the *in vitro* selection field is that these results will help us to optimize and plan future peptide engineering projects to avoid these pitfalls. This project will thereby enhance our ability to use mRNA display to engineer peptide ligands for other novel targets.

2. What was the impact on other disciplines?

Nothing to Report

3. What was the impact on technology transfer?

Nothing to Report

4. What was the impact on society beyond science and technology?

Nothing to Report

E. CHANGES/PROBLEMS:

1. Changes in approach and reasons for change

Nothing to Report

2. Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

3. Changes that had a significant impact on expenditures

Nothing to Report

4. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

5. Significant changes in use or care of human subjects

Nothing to Report

6. Significant changes in use or care of vertebrate animals.

Nothing to Report

unintended and undesirable bias resulting in enrichment of highly hydrophobic sequences that are unlikely to be useful because of their poor water solubility.

Although the project did not meet its intended goals, the impact on the *in vitro* selection field is that these results will help us to optimize and plan future peptide engineering projects to avoid these pitfalls. This project will thereby enhance our ability to use mRNA display to engineer peptide ligands for other novel targets.

2. What was the impact on other disciplines?

Nothing to Report

3. What was the impact on technology transfer?

Nothing to Report

4. What was the impact on society beyond science and technology?

Nothing to Report

E. CHANGES/PROBLEMS:

1. Changes in approach and reasons for change

Nothing to Report

2. Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

3. Changes that had a significant impact on expenditures

Nothing to Report

4. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

5. Significant changes in use or care of human subjects

Nothing to Report

6. Significant changes in use or care of vertebrate animals.

Nothing to Report

7. Significant changes in use of biohazards and/or select agents

Nothing to Report

F. PRODUCTS:

1. Publications, conference papers, and presentations

Nothing to Report

2. Journal publications.

Nothing to Report

3. Books or other non-periodical, one-time publications.

Nothing to Report

4. Other publications, conference papers, and presentations.

Nothing to Report

5. Website(s) or other Internet site(s)

Nothing to Report

6. Technologies or techniques

Nothing to Report

7. Inventions, patent applications, and/or licenses

Nothing to Report

8. Other Products

Nothing to Report

G. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

1. What individuals have worked on the project?

Name:	*Terry T. Takahashi (No Change)*
Project Role:	PI

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Name:	*Richard W. Roberts (No Change)*
Project Role:	*Co-PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Name:	*Dr. Kaori Noridomi*
Project Role:	*Postdoctoral Researcher*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*0.5*
Contribution to Project:	*Dr. Noridomi worked to clone the N-myc protein for expression*
Funding Support:	USC general funds

Name:	Ms. Skyler Brown
Project Role:	*High School Researcher*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*2*
Contribution to Project:	*Ms. Brown worked with Dr. Noridomi to perform the bead-only selections to see their effects on peptide library composition*
Funding Support:	USC SHINE Program

Name:	Ms. Chloe Kim
Project Role:	*High School Researcher*
Researcher Identifier (e.g. ORCID ID):	*N/A*

Nearest person month worked:	*2*
Contribution to Project:	*Ms. Kim worked with Dr. Noridomi to perform the bead-only selections to see their effects on peptide library composition*
Funding Support:	USC SHINE Program

2. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

3. What other organizations were involved as partners?

Nothing to Report

H. SPECIAL REPORTING REQUIREMENTS

1. COLLABORATIVE AWARDS:

Not applicable

2. QUAD CHARTS:

Not applicable

I. APPENDICES:

References Cited

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