CALIFORNIA STATE UNIVERSITY SAN MARCOS

PROJECT SIGNATURE PAGE

PROJECT SUBMITTED IN PARTIAL FULLFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE
MASTER OF SCIENCE

IN

BIOTECHNOLOGY

PROJECT TITLE: A Novel High Throughput Method for the Purification of Peptides under Investigation

AUTHOR: Ross Fellows

DATE OF SUCCESSFUL DEFENSE: 04/15/2014

THE PROJECT HAS BEEN ACCEPTED BY THE PROJECT COMMITTEE IN
PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF
SCIENCE IN BIOTECHNOLOGY.

Dr. Julie Jameson
PROJECT COMMITTEE CHAIR

Dr. Sajith Jayasinghe
PROJECT COMMITTEE MEMBER

Dr. Mack Flinspach
PROJECT COMMITTEE MEMBER
Abstract

A Novel High Throughput Method for the Purification of Peptides under Investigation

Company Sponsor Mack Flinspach, PhD.

Ross Fellows 05-15-2014

Professional Science Masters Program, CSUSM

The pharmaceutical market for peptide drugs is quickly rising, but several technical barriers to the discovery process till exist. Solid phase extraction (SPE) is a simple and crude purification method that can increase throughput and total yields of peptides under investigation and provide a competitive advantage. The standard method for peptide purification is reverse phase HPLC gradient, which regularly yields inadequate amounts for screening. Incorporating the SPE method into our process drastically increases peptide yields, increases throughput, decreases costs, and simplifies the purification. An important benefit of this method is that it can be executed with no sophisticated equipment and with minimal training. The ease of use and ability to process 96 samples in tandem allow more efficient production compared to complicated reverse phase HPLC gradients where only one sample can be run at a time. The small volume of resin used in the SPE format allows for very little organic solvent to be consumed during the process, which decreases costs and environmental waste. The total peptide yield improvement over the current method is 3-fold higher which allows molecules to be screened that would not have been possible with the previous method. The benefits of SPE come with an average of a 4% decrease in total peptide purity, which we have shown has no effect on downstream screening. Overall, the method has shown to be very efficient, reproducible, and enabled screening of peptides that would not have been possible otherwise.
A Novel High Throughput Method for the Purification of Peptides under Investigation

Project Chair: Julie Jameson, PhD
Committee Member: Sajith Jayasinghe, PhD
Committee Member: Mack Flinspach, PhD

Ross Fellows
PSM Symposium
California State University, San Marcos
05-14-14
Highest selling biotech/pharma products represent different therapy platforms

- **Highest selling drug**
  - 2006 sales of ~$13 billion
  - “Small molecule”
  - “Pharmaceutical”

- **#4 selling therapy in 2013**
  - 2013 sales of ~$8 billion
  - “Peptide therapeutic”
  - “Biologic”

- **#1 selling therapy in 2013**
  - 2013 sales of ~$11 billion
  - “Large molecule”
  - “Biologic”
Peptides offer a unique alternative to traditional small/large molecule therapies

**Peptide Therapeutics**
- Superior specificity
- Good safety profile
- Flexible dosing
- Reach new targets
- High cost MFG ($$)

**Small molecules**
- Ideal dosing
- Low cost MFG ($)
- Toxicity issues
- Low specificity

**Monoclonal antibody biologics**
- Superior specificity
- Good safety profile
- High cost MFG ( $$$)
- Ltd. dosing options
Peptide therapeutics entering the clinic per year per decade are drastically increasing

Since the 1980s, peptides have been studied as treatments for a wide variety of indications. During 2000-2008, peptides entering study were most frequently treatments for cancer and metabolic disorders (18% and 17%, respectively). The percentage of candidates for metabolic disorders represents a notable increase from the 1980s and 1990s, when 2% and 11% of peptide therapeutics, respectively, were studied in this category. Indications such as diabetes, obesity, and osteoporosis are included in the metabolic category. Decreases were observed in the study of peptides as treatments for allergy and immunological disorders, as well as for cardiovascular disease.

Identifying potential peptide drug candidates can be very challenging

- **Goal**: Identify drug candidates from tens of thousands of peptides

- **Challenge**: Current high throughput peptide purification is time consuming and produces very low amounts

- **Solution**: Find alternative method that will allow for sufficient quantities to be tested and candidates identified
The current standard method of peptide purification is inefficient and insufficient.

Dionex HPLC System

- Complicated equipment $$$
- Need highly trained employee $$
- Purifies one at a time at 1 hr each $
- Uses large amounts of solvent (ACN) $

Typical HPLC Chromatogram

- Avg. pure peptide ~6.3 ug
- 20 ug needed for testing
What is solid phase extraction (SPE) ?

Solid-phase extraction (SPE) is a separation process by which compounds that are dissolved in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties.

Environmental

“Solid Phase Extraction / HPLC Analysis of Acidic Herbicides In Drinking Water”

Food/Agriculture

“Solid Phase Extraction of Pesticides from Fruits and Vegetables, for HPLC..”

Biotech/Pharma

“Extraction of Amphetamine and Related Drugs from Urine using SPE”

SPE cartridge method adapted to 96 well block format to increase throughput

- Vacuum manifold to speed process
- 96 samples purified in tandem – Ideal for high throughput
Peptide-X, Y, Z yields are roughly 3-fold higher for samples purified by SPE method vs. the RP-HPLC method

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Peptide Yield (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RP-HPLC</td>
</tr>
<tr>
<td>Peptide-X</td>
<td>7.8</td>
</tr>
<tr>
<td>Peptide-Y</td>
<td>13.4</td>
</tr>
<tr>
<td>Peptide-Z</td>
<td>10.2</td>
</tr>
<tr>
<td><strong>Avg. Yield</strong></td>
<td><strong>10.5 ug</strong></td>
</tr>
</tbody>
</table>

- Identical samples purified by each method
- Average yield of 3.4x more peptide with SPE method
Peptide-X,-Y,-Z purity evaluated by analytical RP-HPLC

Avg. peptide purity of –X, -Y, -Z differ by 4%

<table>
<thead>
<tr>
<th>Peptide</th>
<th>RP-HPLC</th>
<th>SPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide-X</td>
<td>93</td>
<td>89</td>
</tr>
<tr>
<td>Peptide-Y</td>
<td>95</td>
<td>90</td>
</tr>
<tr>
<td>Peptide-Z</td>
<td>92</td>
<td>89</td>
</tr>
</tbody>
</table>

Avg. Purity 93% 89%

Analytical chromatograms overlay of –X purified by SPE and RP-HPLC

Peptide-X

--RP-H PLC, 93% Purity
--SPE, 89% Purity
Peptide-X purified by different methods shows reproducible activity

*Work done by colleague Rebecca Hagan*
The SPE method drastically improves yields for 50 samples tested by either method.

*The SPE method allows many more peptide samples to be screened that would not have been possible otherwise.*
Summary of advantages of using SPE method for production of peptides of interest

**Simplicity**
- RP-HPLC – Complicated HPLC equipment and highly trained staff
- SPE – No equipment or training necessary

**Speed**
- RP-HPLC – 1 sample/hour
- SPE – 96 samples/2-3 hours

**Efficiency**
- Increases peptide yields at least 3-fold
- Saves $$$

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![SPE Fold Increase vs. RP-HPLC Purification](chart.png)
Thank you!

- Dr. Julie Jameson
- Dr. Betsy Read
- Dr. Sajith Jayasinghe
- Dr. Mack Flinspach
- Dr. Alan Wickenden
- Rebecca Hagan

PSM Biotechnology Cohort 4 (Congrats!!)
Questions?