# Protease Identification by Combined Fluorescent Zymography and Proteomics

*Amanda Ferrante[1], Alyssa Murphy[1], Samuel Rozans[1], Abdul-Nafea Syed[1], E. Thomas Pashuck[1]*

*[1]Department of Bioengineering at Lehigh University, Bethlehem, PA, 18015 USA*

## Introduction

- Engineering biological tissues for regenerative environments is difficult because each cell can have their own physiological requirements
- Cell-secreted proteases: a promising method for creating a local biomaterial response to a specific cell type
- Cell-specific protease substrate peptides can be identified using a proteomics-based approach; however, this necessitates a method for identifying the proteases cleaving the peptides

## Fluorogenic Fmoc Solid Phase

- Peptides were synthesized with fluorescein and DABCYL as a quencher
- Utilized an azide functional group to covalently couple the peptide to the hydrogel
- The PanMMP (KGPQGIWGQK) (Fig. 1) peptide was synthesized, purified, and its mass was validated using LCMS

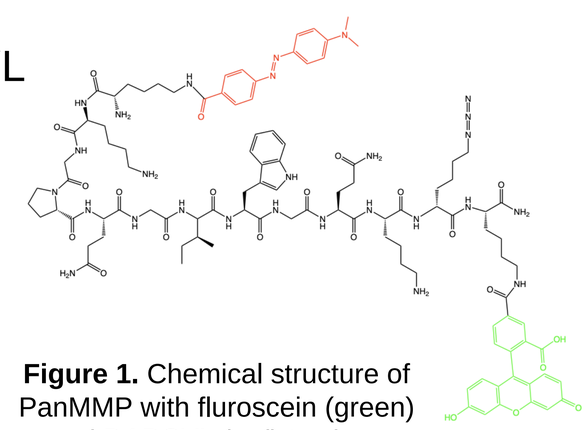

**Figure 1.** Chemical structure of PanMMP with fluroscein (green) and DABCYL (red) to show fluorescence with an azide for conjugation to the hydrogel

## Cell Culture

- Human monocytic cell line was cultured for 48 hours
- Media was then collected, concentrated with a 10 kDA centrifugal filter, and loaded into wells of multilayer polyacrylamide gel

## Fluorescent Zymography Gel Fabrication

- Gels contain a 10% Polyacrylamide Resolving Gel with a quenched, fluorescent PanMMP peptide coupled to the hydrogel with an azide cross-linker and a 5% Polyacrylamide Stacking Gel (Fig. 2)
- Incorporated equivalents of 30 µg of protein per well[2]

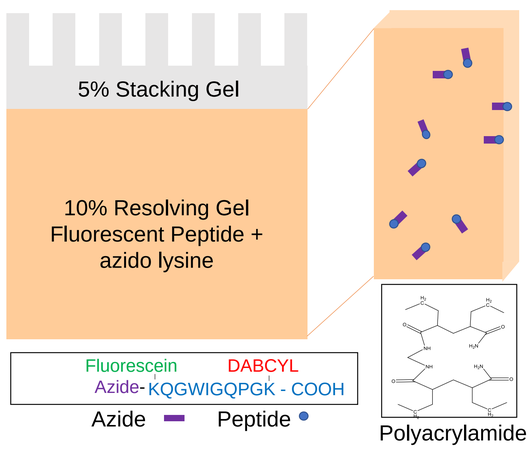


**Figure 2.** Schematic diagram of multi-layer polyacrylamide gel. The azido lysine cross-links the fluorescent peptide to the polyacrylamide.

## Fluorescent Zymography

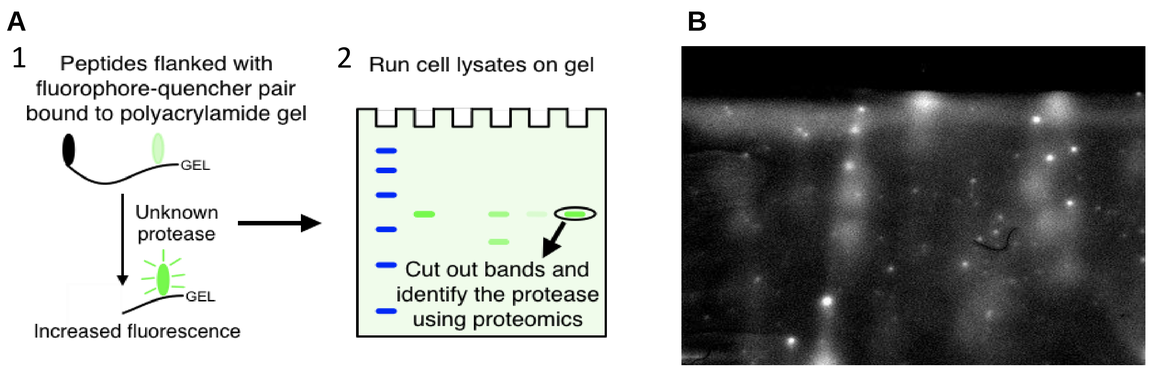


**Figure 3.** (A) A schematic of the protease identification protocol and (B) Fluorescent trypsin bands found in the PAGE gel. Gel was imaged using the Gel Doc XR+ Gel Documentation System and adjusted for contrast with ImageJ.

- Cleavage of cell lysate by a protease causes increase in fluorescence (Fig. 3A)
- The protease trypsin was added to all wells in hydrogel for use as a positive control to validate a protease induced increase in fluorescence (Fig. 3B)

## Liquid Chromatography – Mass Spectrometry

| MMPs | Peptides Identified |
|---|---|
| MMP-12 | EAAYEIEAR+EKNNVLFGER |
| MMP-10 | HTLGFPPTIR |

**Table 1.** After performing LCMS on gel samples, peptides were identified for MMP-12 and MMP-10 with high confidence.

- Peptides corresponding to MMP-12 and MMP-10 were identified with high confidence
- This indicates that a proteomics-based approach can be taken to identify proteins that are present after zymographic separation

## Conclusions and Future Work

- This work presents a promising platform for identifying the proteases responsible for peptide cleavage through the incorporation of protease-degradable peptides
- Future work will incorporate cell-specific protease substrate peptides that are cleave by unknown proteases

## Acknowledgements

- AF acknowledges the Clare Boothe Luce Research Scholarship funded through the Henry Luce Foundation
- AF acknowledges the David and Lorraine Freed Undergraduate Research Symposium at Lehigh University

LEHIGH UNIVERSITY

***References*: [1] Pashuck, *Methods Molecular Biology* 2018, 1758:177-192; [2] Deshmukh et al, J. Vis. Exp 2019, 143**

***https://www.pashucklab.com/***