# Synthesis of Tunable Protein-Polymersome Conjugates for Enhanced Targeting of Cancerous Tissues


Lauren A. Hubert[3], Blaise R. Kimmel, Ph.D.[1], Payton T. Stone[1], Hayden M. Pagendarm[2], John T. Wilson, Ph.D.[1,2]

[1]*Department of Chemical and Biomolecular Engineering, Vanderbilt University;* [2]*Department of Biomedical Engineering, Vanderbilt University*

[3]*Department of Chemical Engineering, University of Rhode Island*


## Introduction

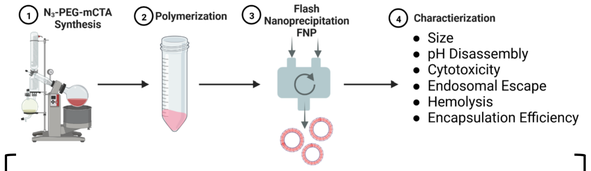
**The Background:** Polymersomes have no inherent systemic targeting or autoregulation of biodistribution. A fluorescent protein can provide a way to image the location of a polymersome, and an antibody fragment will ensure it goes to the right cells.

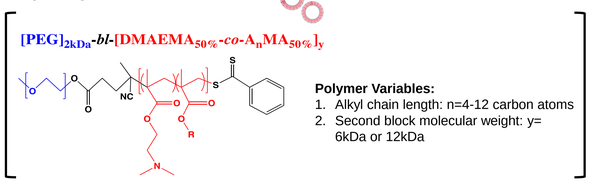
**The Problem:** Traditional modalities for adding proteins to polymersomes are difficult to perform and lack control over location and quantity of added protein. These variables all influence protein functionality.

**The Goal:** Use selective conjugation of a small molecule (BCN) to an aGD2-mCherry fusion protein in order to click on an azide linked polymersome. This creates a platform technology to produce enhanced protein-polymersome conjugates.

## Methods

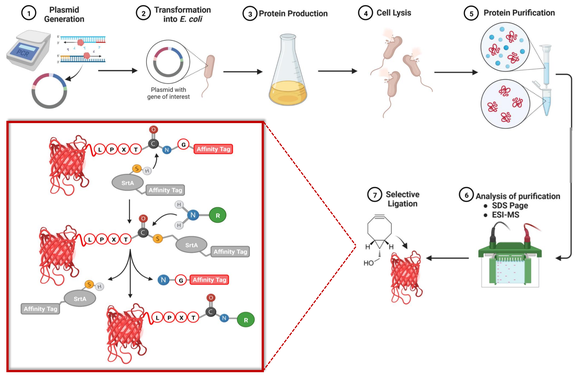
### 1. Creation of the Polymersomes



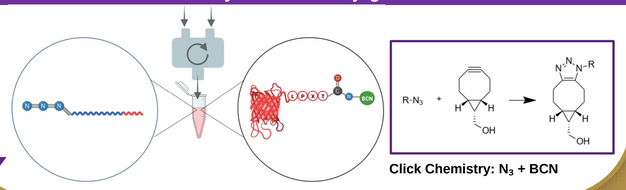




**Polymer Variables:**
1. Alkyl chain length: n=4-12 carbon atoms
2. Second block molecular weight: y= 6kDa or 12kDa

### 2. Creation of the Proteins




### 3. Creation of the Protein-Polymersome Conjugate




## Results: Polymer Library & Azide-Linked Polymersome

| Polymer | Specifications |
|---|---|
| 1 | E-4-40-6k |
| 2 | E-4-40-12k |
| 3 | E-4-40-20k |
| 4 | D-4-50-6k |
| 5 | D-6-50-6k |
| 6 | D-8-50-6k |
| 7 | D-10-50-6k |
| 8 | D-12-50-6k |
| 9 | D-4-50-12k |
| 10 | D-6-50-12k |
| 11 | D-8-50-12k |
| 12 | D-10-50-12k |
| 13 | D-12-50-12k |

**Polymer Library Key: [E/D]-x-y-z**
**[E/D]** = E(DEAEMA) / D(DMAEMA)
**-x** = Alkyl Chain Length
**-y** = Alkyl Chain Composition
**-z** = 2nd Block molecular weight

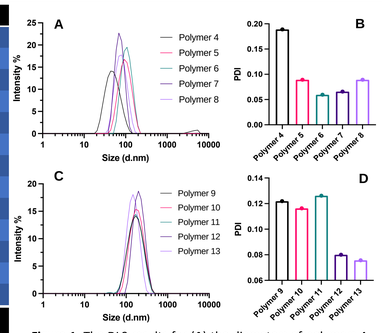



**Figure 1.** The DLS results for (**A**) the diameters of polymers 4 through 8 with their respective (**B**) polydispersity indices (PDI), and (**C**) the diameters of polymers 9 through 13 with their respective (**D**) PDIs.

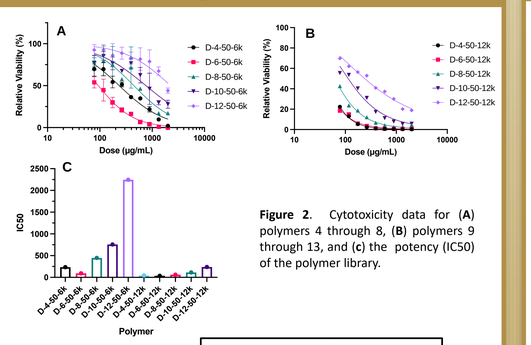
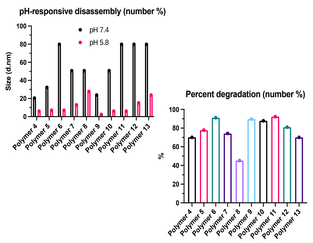
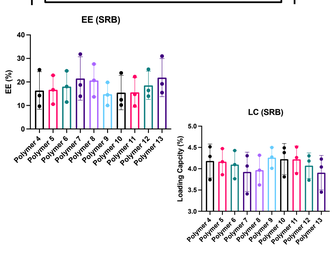



**Figure 2.** Cytotoxicity data for (**A**) polymers 4 through 8, (**B**) polymers 9 through 13, and (**c**) the potency (IC50) of the polymer library.

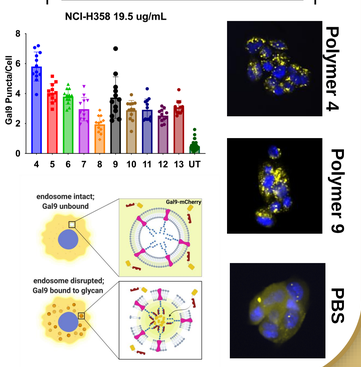
### pH Disassembly Study




### Model Drug Loading




### Endosomal Escape




## Results: Protein Purification

### Pure Protein Verification

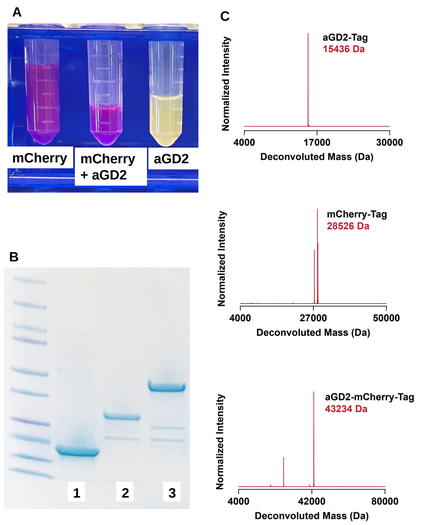
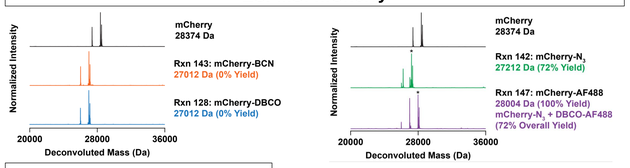
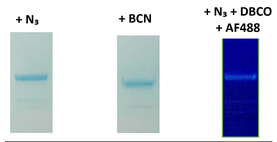



**Figure 1.** (**A**) Image of protein samples (**B**) SDS Page gel verification of 1. raw aGD2, 2. purified mCherry, and 3. purified aGD2 and mCherry fusion (**C**) ESI-MS verification of 1. raw aGD2, 2. purified mCherry, and 3. purified aGD2 and mCherry fusion

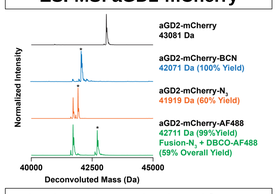
### ESI-MS: mCherry



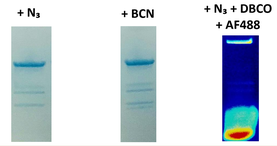

### SDS-PAGE: mCherry

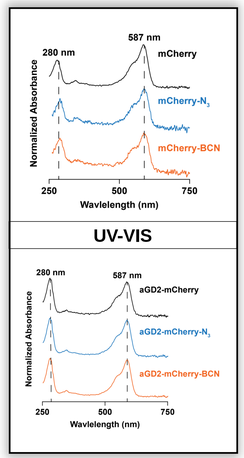



### ESI-MS: aGD2-mCherry

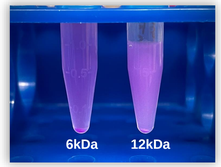



### SDS-PAGE: aGD2-mCherry

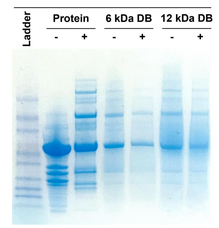



## Conclusion: Protein-Polymersome Conjugate

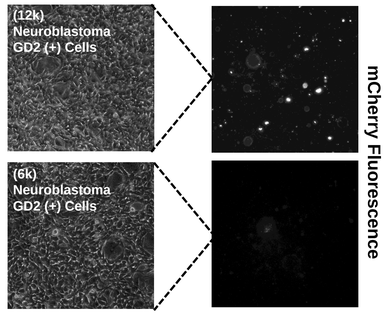



### SDS-PAGE: Gel Verification

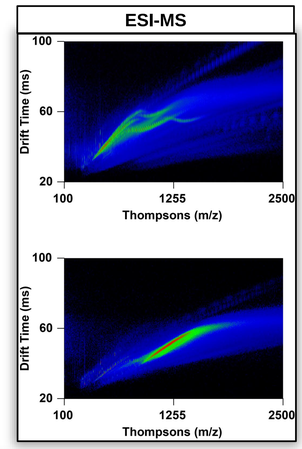



### Flow Cytometry



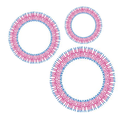

### ESI-MS




## Future Directions

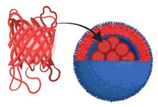
✓*In Vivo* pilot study imaging the fluorescence of mCherry as a method to track the biodistribution of the protein-polymersome conjugate.

✓Test a 20kDa second block polymer which may lead to a new polymer composition for creating improved nanocarriers.



✓Creating protein-polymersome conjugates using different polymers from the original 13 polymer library, and changing the ratio of protein concentration to polymer concentration during FNP to optimize the conjugation reaction efficiency

✓Using flash nanoprecipitation to encapsulate mCherry or the mCherry-aGD2 fusion protein in order to enable cytosolic delivery of proteins.



## Acknowledgements


- **This research was funded by the National Science Foundation (Grant: NSF-DMR 1852157) and the NSF CAREER award (CBET 1554623)**
- **Graphics created with BioRender.com**
- **Special thanks to VINSE and the members of the Wilson Lab, especially Dr. John Wilson, Blaise Kimmel, and Payton Stone**