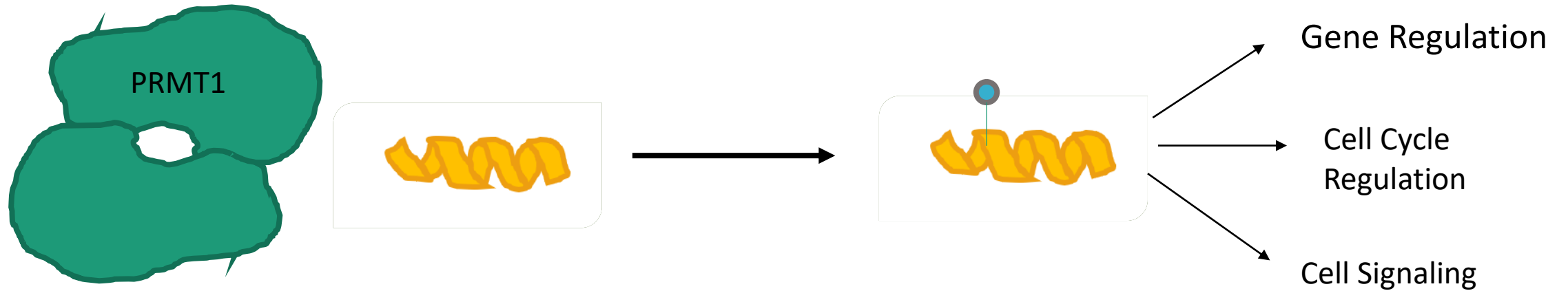


Investigating Position 312 in PRMT1 for Future Fluorescent Labeling Studies

Sarah Nielson

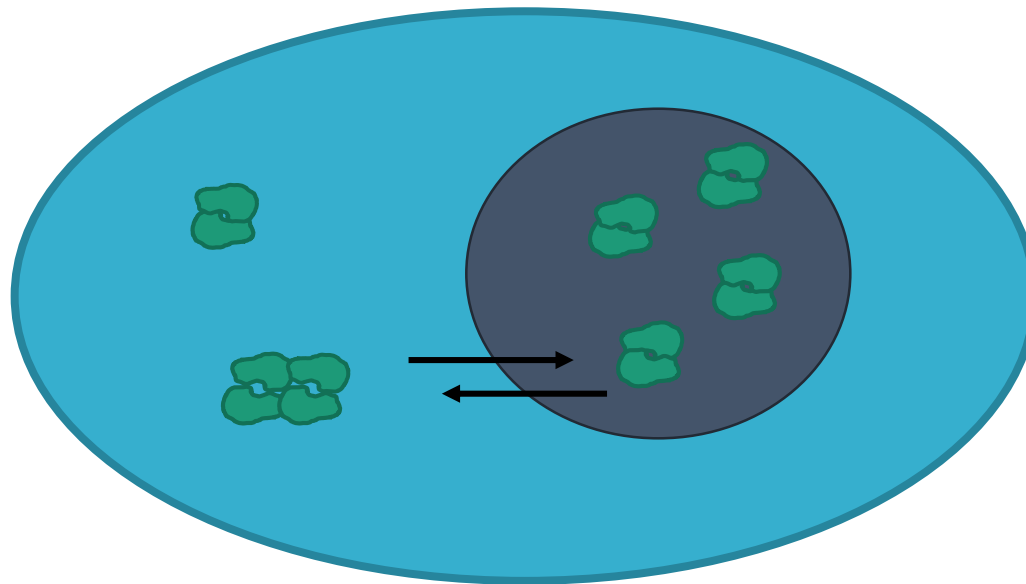
PRMT1 Regulates Cellular Health



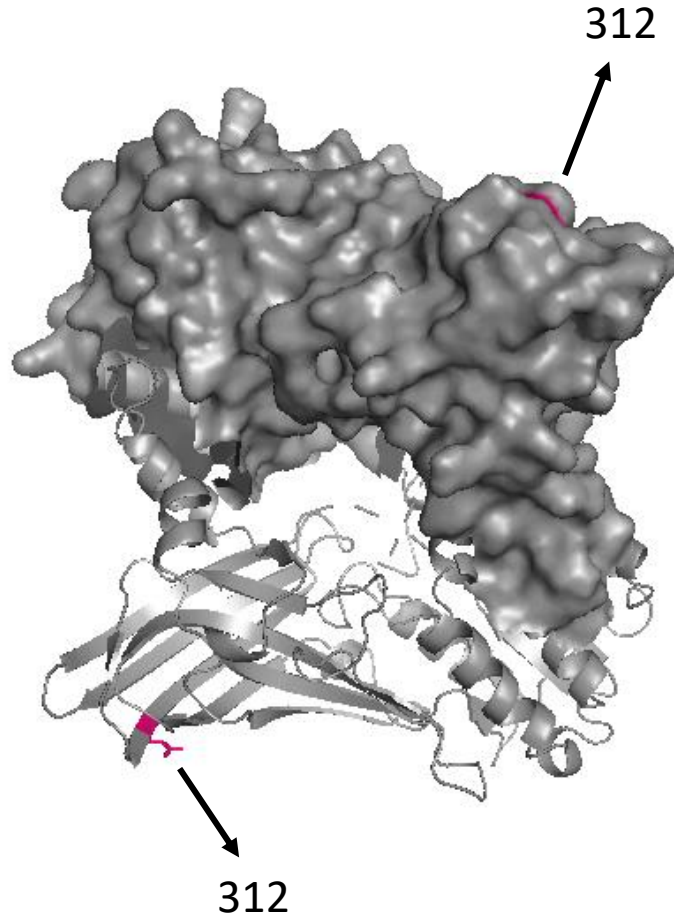
PRMT1 post-translationally methylates protein substrates. Arginine methylation can regulate a diversity of cellular processes.

Visualization of PRMT1 *in vivo*

- There are still several unknowns about PRMT1's subcellular location and oligomeric state *in vivo*



E312C Octamutant Humanized rPRMT1



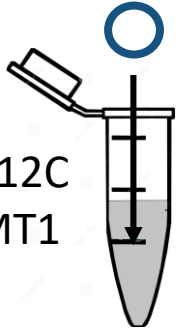
- Position 312 is not found in the dimer or tetramer interface
- Surface-Exposed
- Position 312 is located at the end of a fixed beta sheet

Project Aims

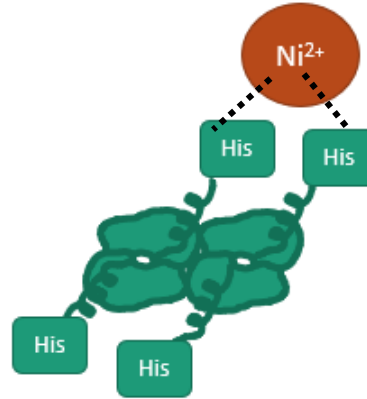
1. To design, express, and purify E312C Octamutant Humanized rPRMT1
2. To label the E312C PRMT1 construct with Alexa Fluor 488 Maleimide
3. To test the activity of the E312C PRMT1 construct before and after labeling

Methodology

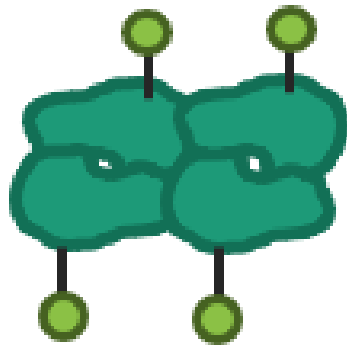
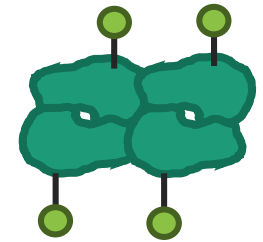
Recombinant
Expression of E312C
Octamutant PRMT1
in *E. coli*



Protein
Purification
through Nickel
Chromatography



Labeling
E312C
Octamutant
PRMT1
with Alexa
Fluor 488
Maleimide

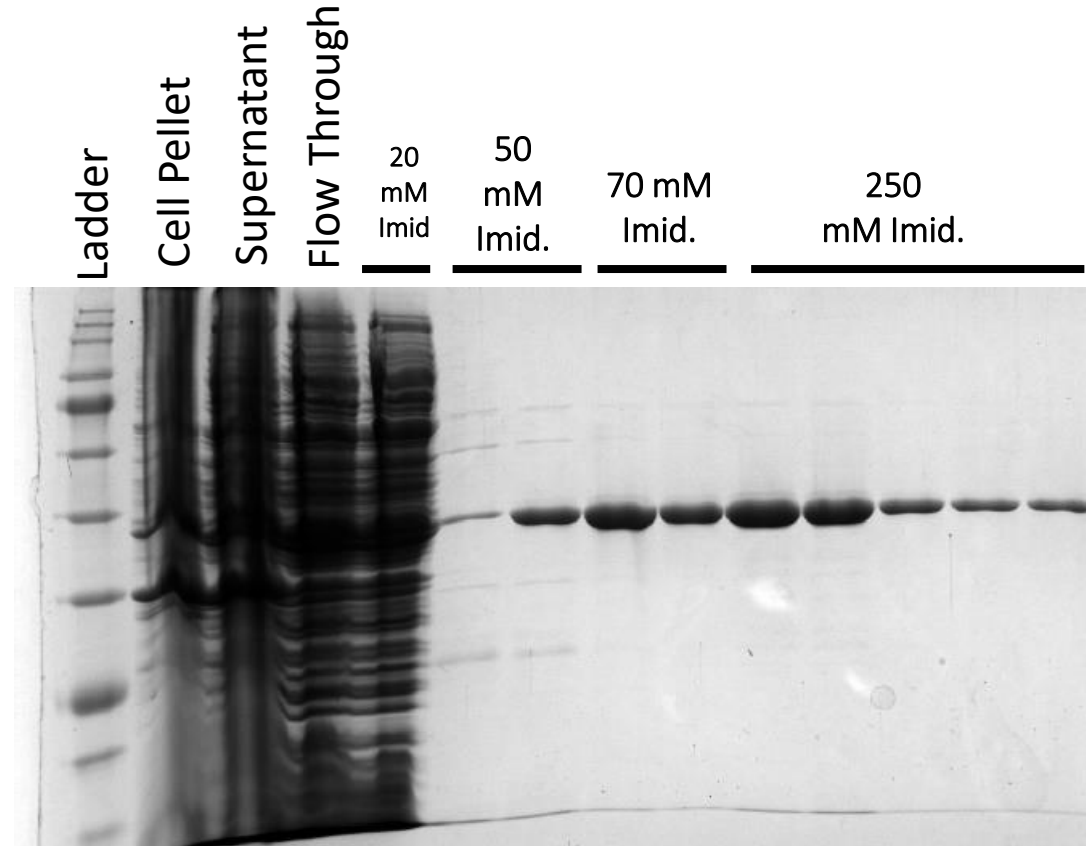
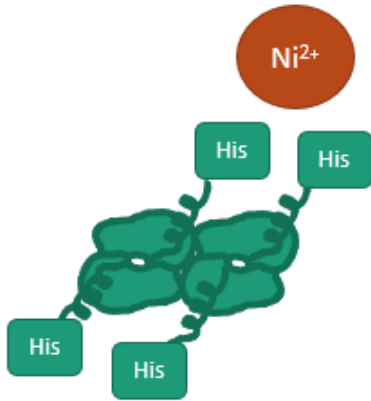


Determine Degree of Labeling

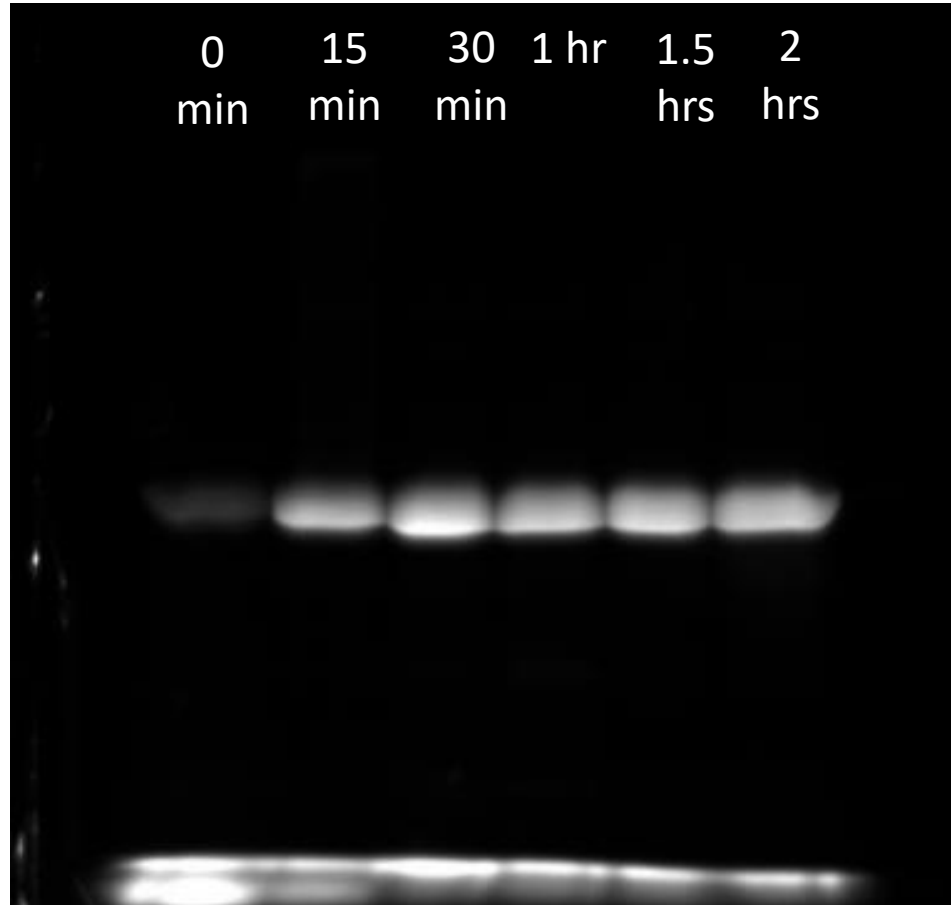
Assess Oligomeric State through Native Page

Perform Activity Assays

Protein Purification through Nickel Chromatography

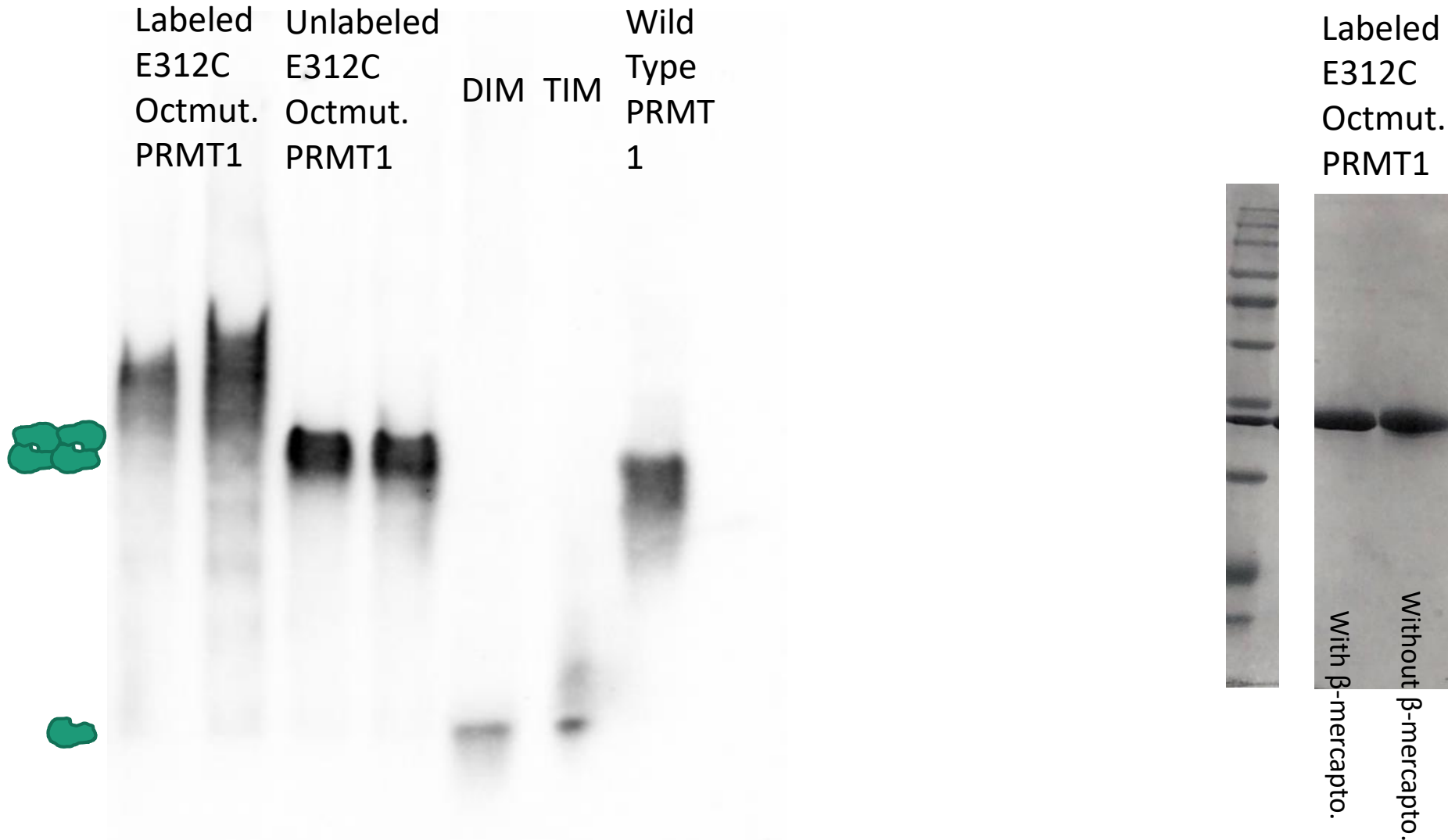


Labeling Reaction at Different Time Points



- At 45 minutes, the degree of labeling was found to be 0.91 moles of dye to 1 mole of protein

Oligomeric State of E312C Octamutant PRMT1



Analyzing Activity of Labeled and Unlabeled E312C Octamutant PRMT1

