EVALUATION OF DTT AS A CONTAMINANT IN PEPTIDE MAPPING

REBECCA CHAN

DISCLAIMER:

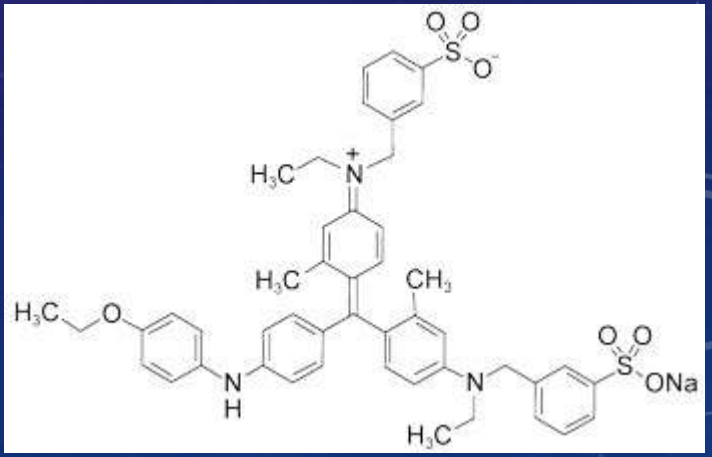
The following information is the opinion of the author and is not affiliated with the US Navy, Army, Air Force, or Government.

CHRONOLOGY

- Week of June 6 – August 26th
- Early June: Lab Safety Trainings
- June-Early August: Spent working on Two Government Projects
  - 1: Radiation
  - 2: Fish Proteins
- August 8- August 26th: Evaluation of DTT as a Contaminant in Peptide Mapping

BASIC SUMMARY:

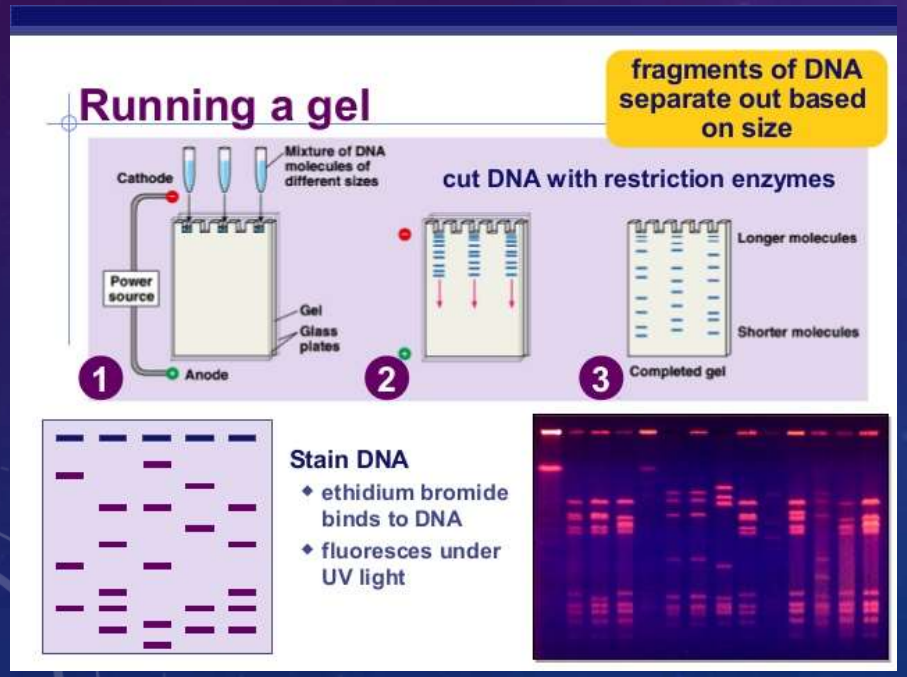
- Peptide mapping:
  - A technique that is frequently used to identify unknown proteins.
  - And we can use mass spectrometry to find those proteins!
- Proteins are polymers of amino acids
  - They do all sorts of incredible things.
    - Give structure to living things
    - Support the immune system
    - Aid chemical reactions



An example of a protein structure

PROCEDURES LEARNED:

- SDS Western Blotting
- Gel Electrophoresis
- In-gel trypsin digests



MAIN EQUIPMENT USED:

METHODS

- In-gel trypsin digests were performed in 100 mM ammonium bicarbonate, pH 8, with 10% v/v acetonitrile, and 20 mM DTT at 37C overnight.
- The supernatant was injected directly onto a Waters C18 column eluted a 0.3 ml/min with a linear gradient of 7.5 to 35% acetonitrile against 0.1% formic acid in water over 30 (Fig. 2) or 90 (Fig. 1) minutes monitoring absorbance at 254 nm and detecting positive ions from 400-3000 m/z with a Shimadzu ion trap time of flight mass spectrometer.

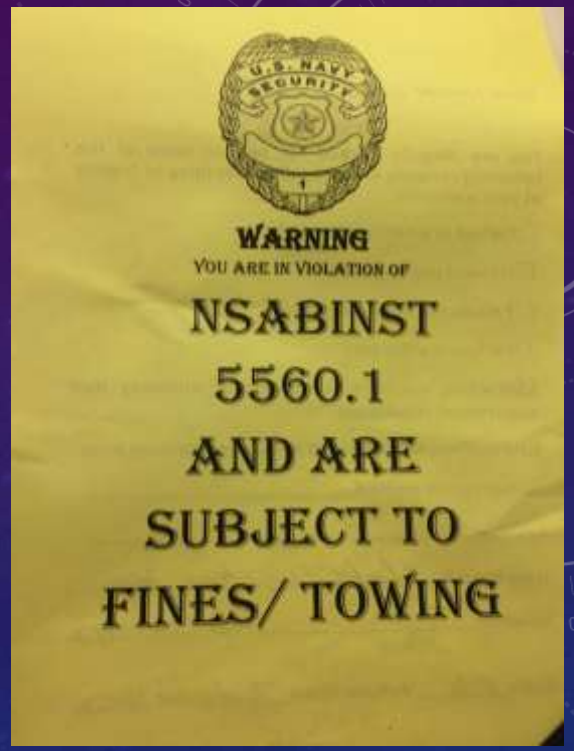
CONCLUSION OF MY LAB:

The trypsin digestion resulted in peaks that did not correspond to peptide proteins

- However, there was material eluting in blank runs
- I concluded that dithiothreitol (DTT) was responsible for the artifacts seen in the blank run
- The contaminant blocks the classification of proteins

WHAT I LEARNED

- DON’T EVER TRY TO PARK AT WALTER REED
- Always ask questions and ask for help
- It’s usually OK to make mistakes
- Mad-pipetting skills courtesy of MMB, reinforced by my lab
- Dealing with difficult equipment without even knowing their names
- I don’t want to work in a lab for the rest of my life



ACKNOWLEDGMENTS

- Red Cross Volunteer program at Walter Reed
- Department of Research Programs
- Holton Arms

American Red Cross

ANY QUESTIONS?