Carrie Stemple

Mentored by Dr. Amy Xu

Characterizing Protein-Adjuvant Interactions in Vaccines

Overview

- Project
- What is in a Vaccine
- Protein Adjuvant Interactions
- Effects of Freeze Thaw Cycles on Adjuvants
- Effects of Freeze Thaw Cycles on Microstructure of Adjuvant and Complexes
- Summary
- Future Research/ Acknowledgements

Project

- Define the interactions between proteins and adjuvants – Binding Isotherm
- Determine the effects of freeze thaw cycles on adjuvants and their interactions – Binding Isotherms, Microscopy, and Dynamic Light Scattering
- Develop understanding of how freeze thaw cycles affect microstructures of proteins, adjuvants, and complexes – Small Angle Neutron Scattering

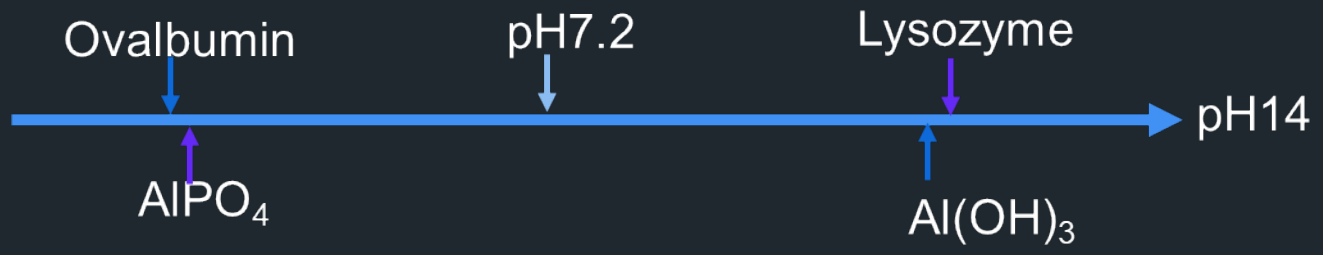
4 Vaccines

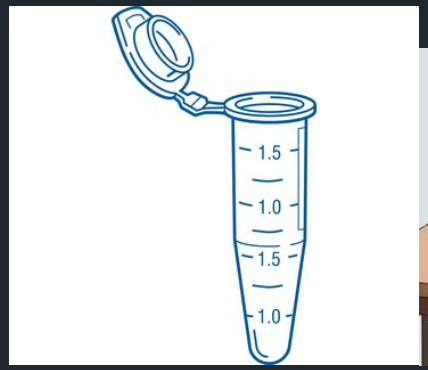
- Purpose
- Components:
  - Antigen
  - Adjuvant
  - Excipients

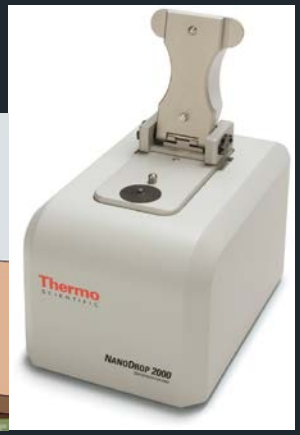
https://www.brenntag.com/biosector/en/products/adju-phos/index.jsp http://www.haoranbio.com/pic/product/2015-03/20150320024234346506.jpg https://www.indiatvnews.com/lifestyle/news-new-vaccine-developed-to-cure-ebola-387386

5 Protein Adjuvant Interactions

- Experiment







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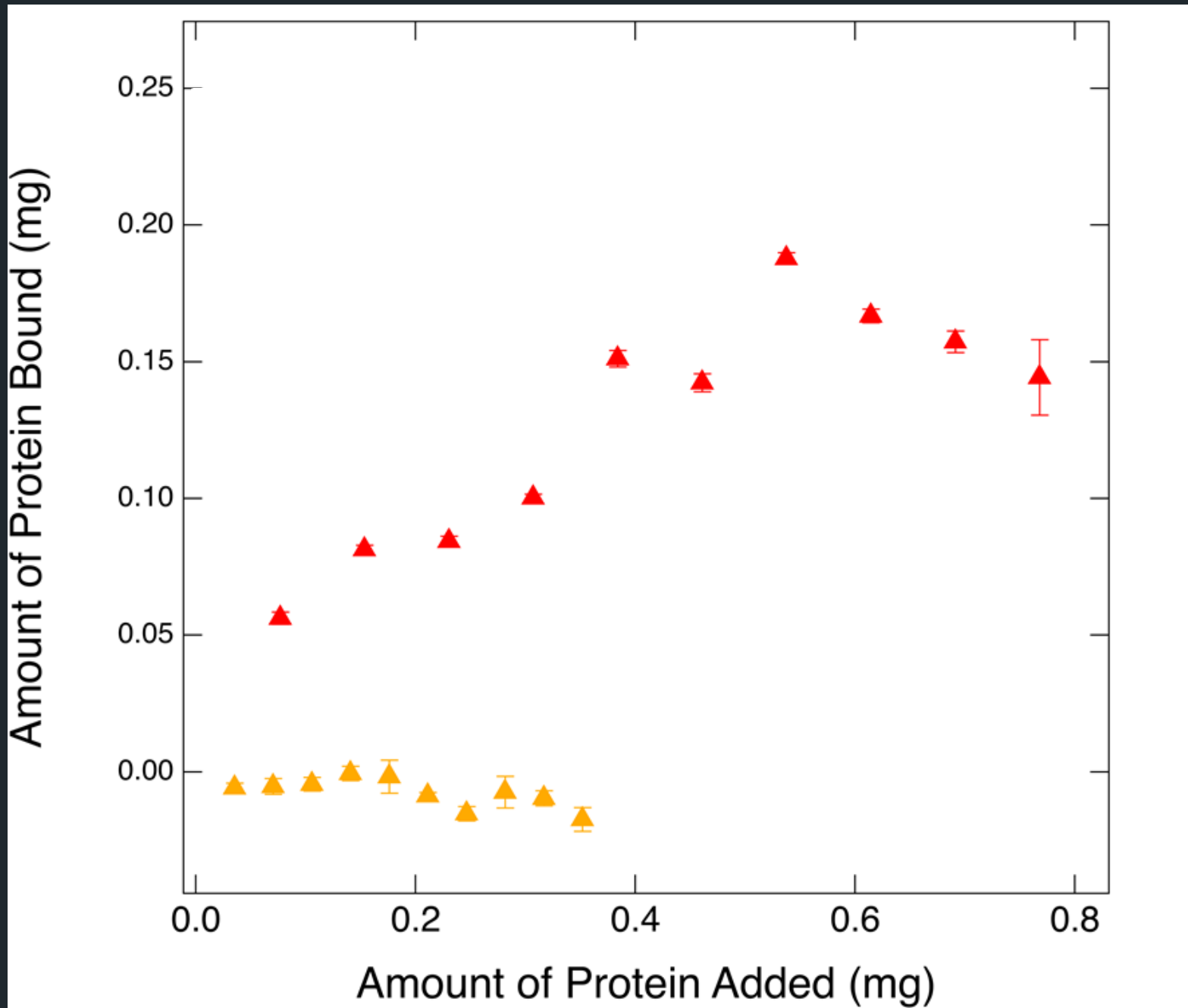
6 Protein Adjuvant Interactions: Binding Isotherm

Aluminum Hydroxide + Ovalbumin

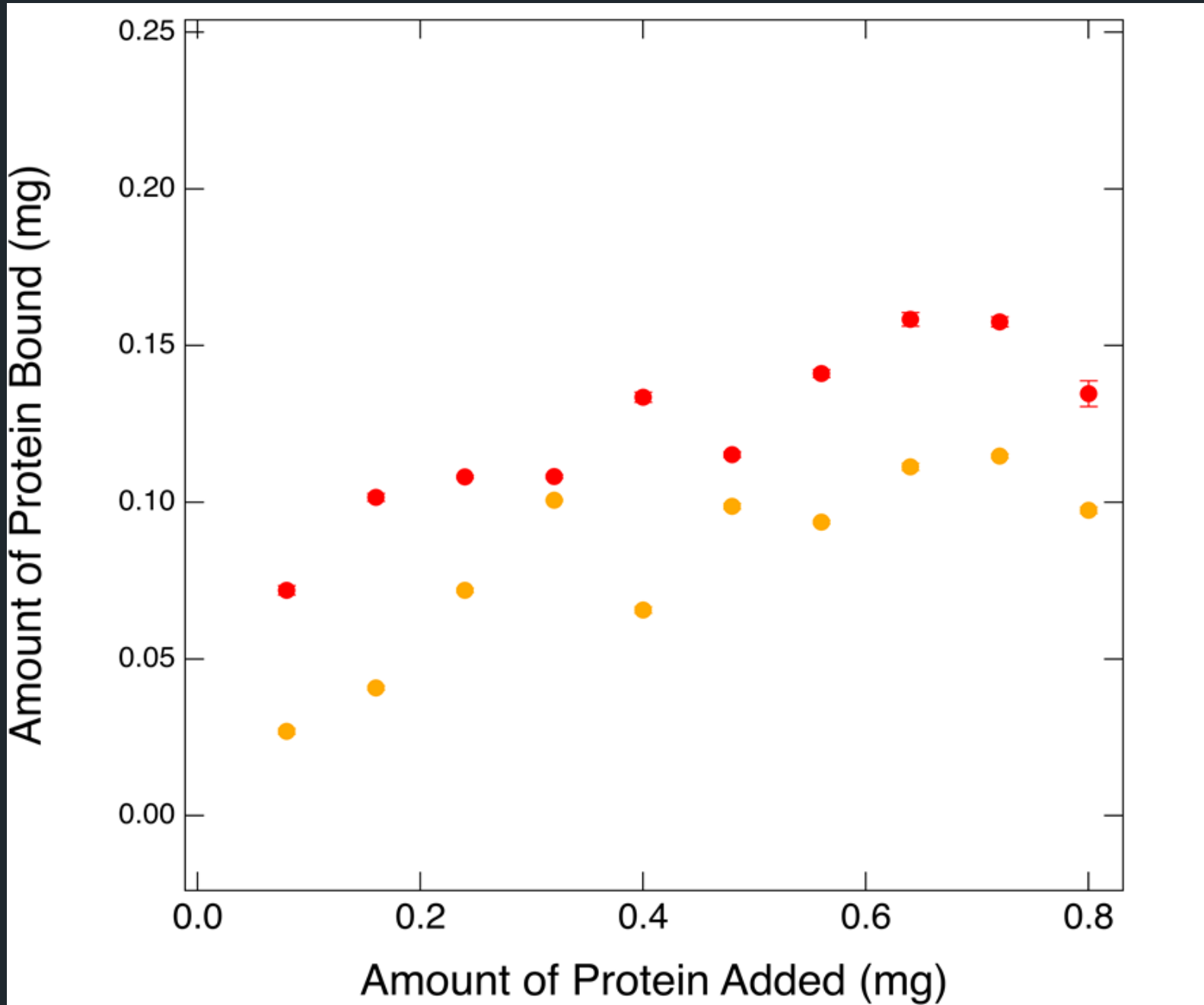
Aluminum Phosphate + Ovalbumin



7 Protein Adjuvant Interactions: Binding Isotherm

Aluminum Hydroxide + Lysozyme

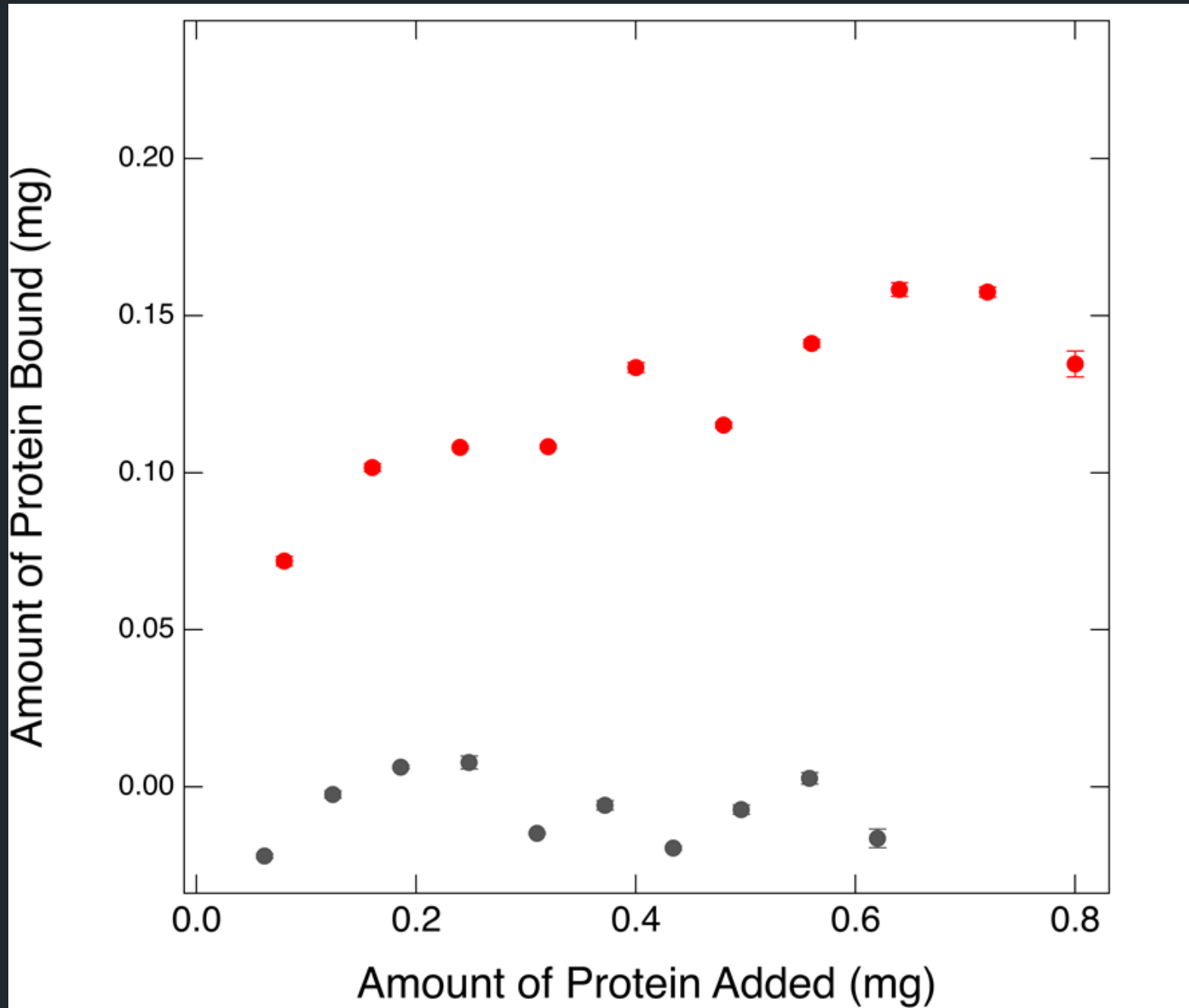
Aluminum Phosphate + Lysozyme



Protein Adjuvant Interactions: Binding Isotherm

Aluminum Hydroxide + Lysozyme in Phosphate Buffer

Aluminum Hydroxide + Lysozyme in Sodium Chloride Buffer



9 Effects of Freeze Thaw Cycles on the Adjuvants

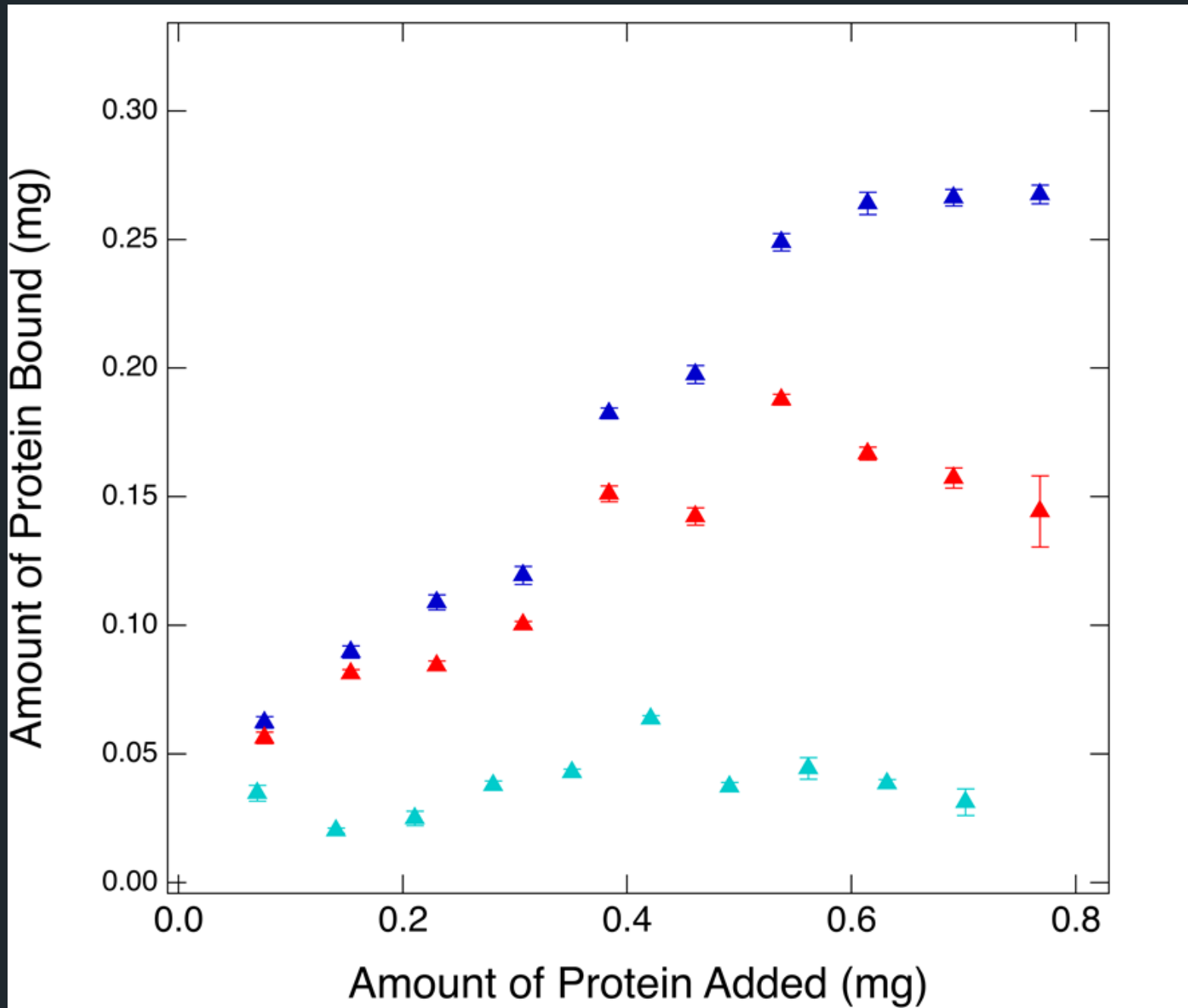
- Importance of Freeze Thaw Cycles
- Experiments

Effects of Freeze Thaw Cycles on Adjuvants: Binding Isotherms

FT Aluminum Hydroxide & Ovalbumin Complex

Fresh Aluminum Hydroxide & Ovalbumin Complex

FT Aluminum Hydroxide + Ovalbumin

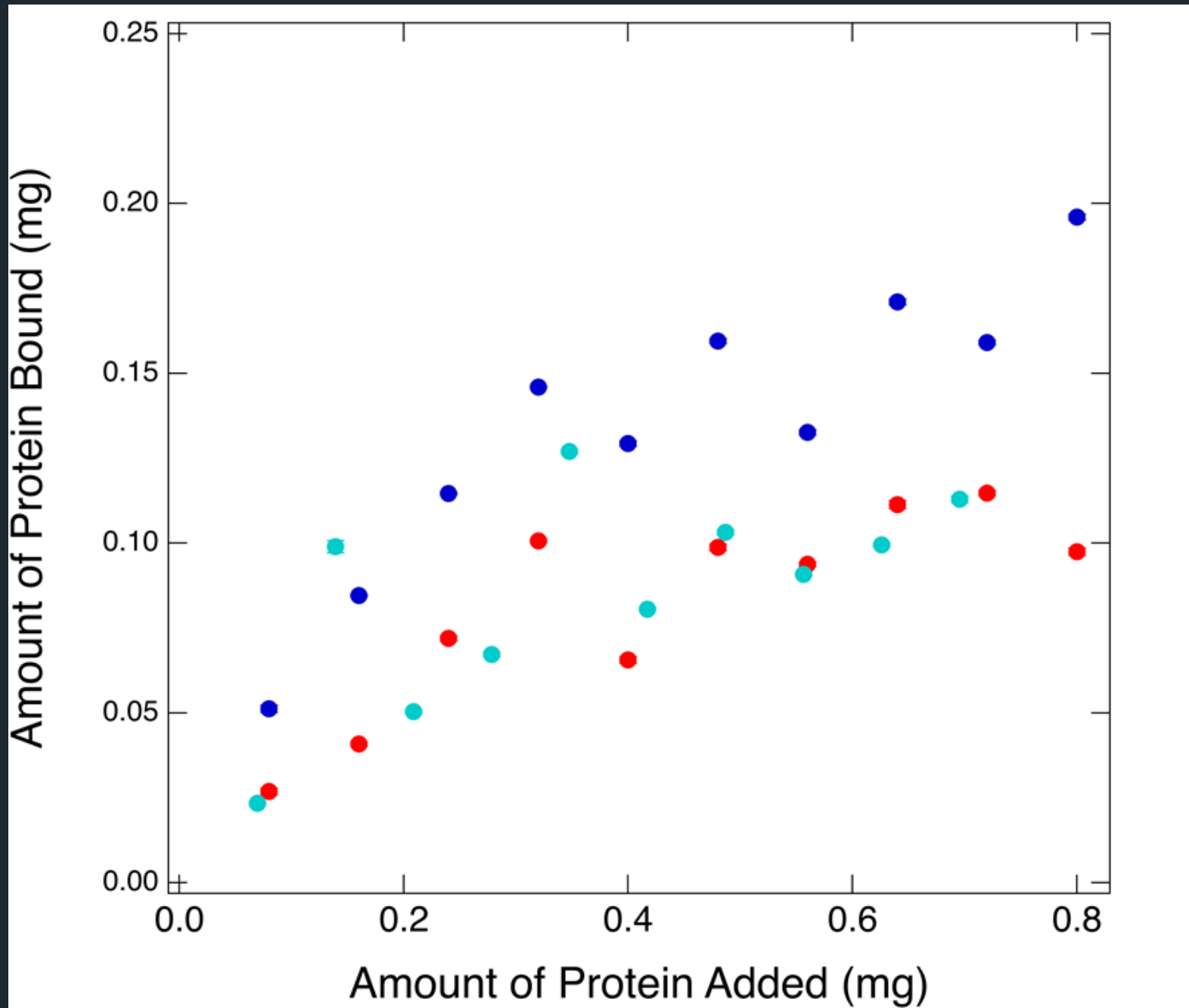


Effects of Freeze Thaw Cycles on Adjuvants : Binding Isotherms

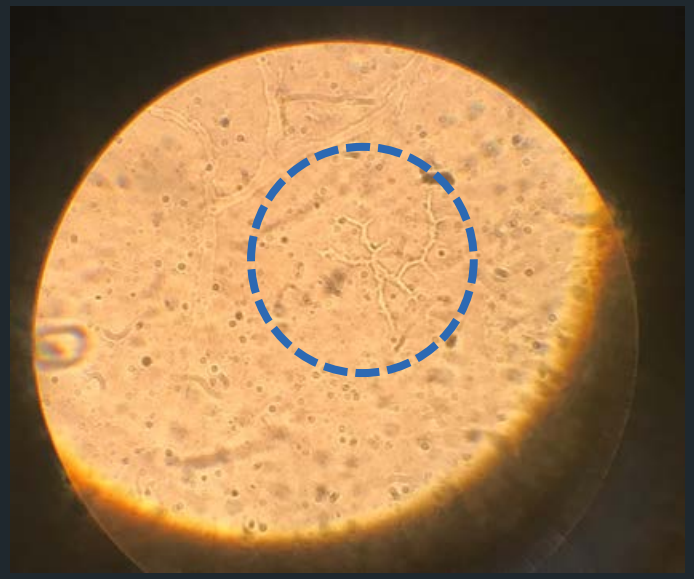
FT Aluminum Phosphate & Lysozyme Complex

Fresh Aluminum Phosphate & Lysozyme Complex

FT Aluminum Phosphate + Lysozyme



Effects of Freeze Thaw Cycles on Adjuvants: Microscopy 40x Magnification



Aluminum Hydroxide: Fresh

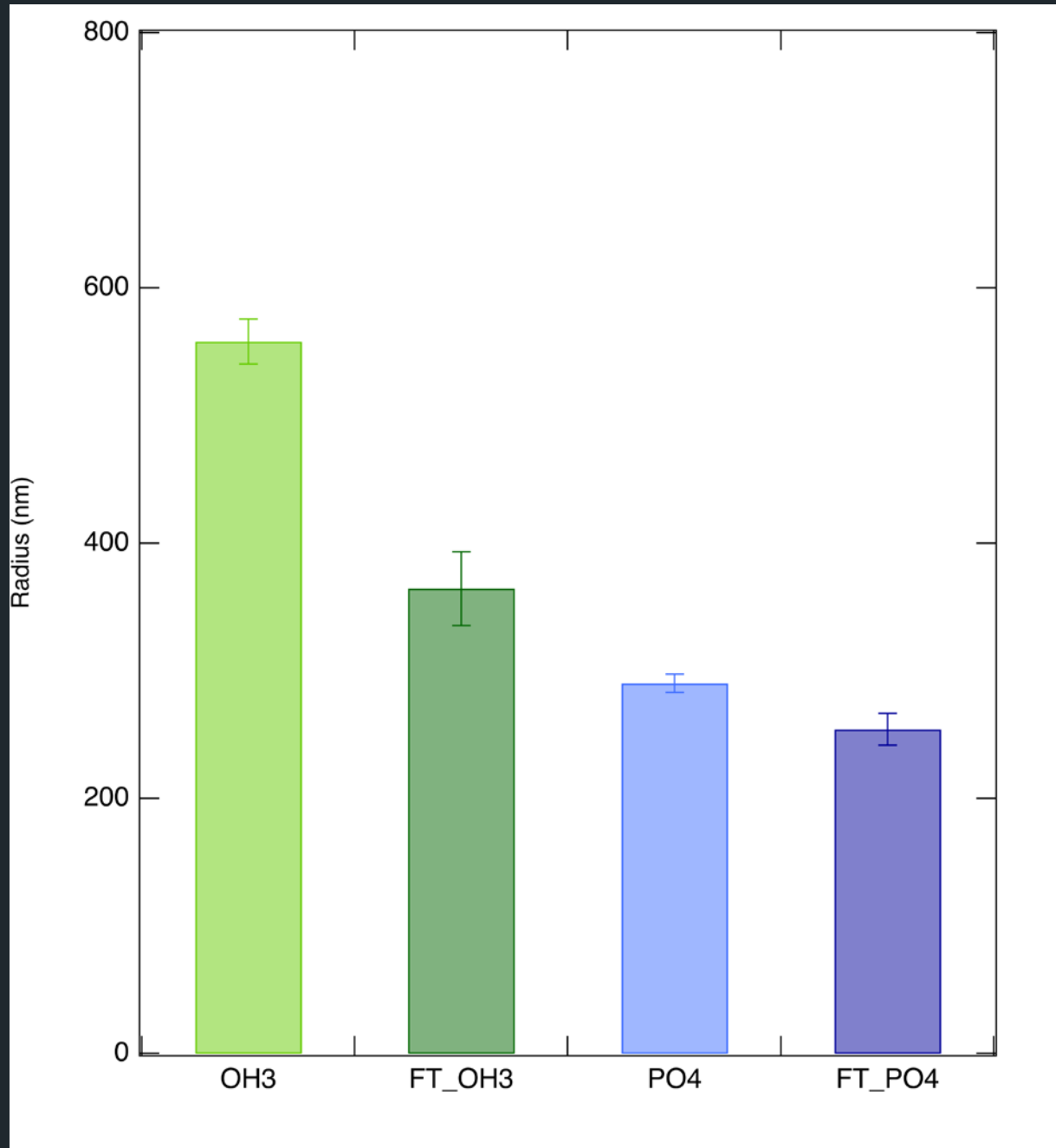
Aluminum Phosphate: Fresh

Aluminum Hydroxide: Freeze-Thawed

Aluminum Phosphate: Freeze-Thawed

Effects of Freeze Thaw Cycles on Adjuvants: Dynamic Light Scattering

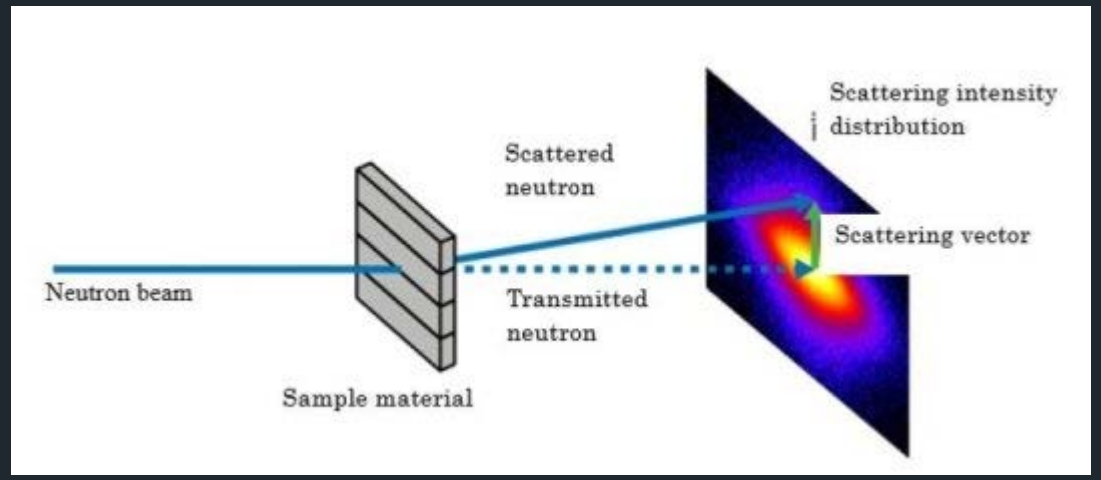
Fresh Aluminum Hydroxide
FT Aluminum Hydroxide
Fresh Aluminum Phosphate
FT Aluminum Phosphate



Effects of Freeze Thaw Cycles on Microstructures

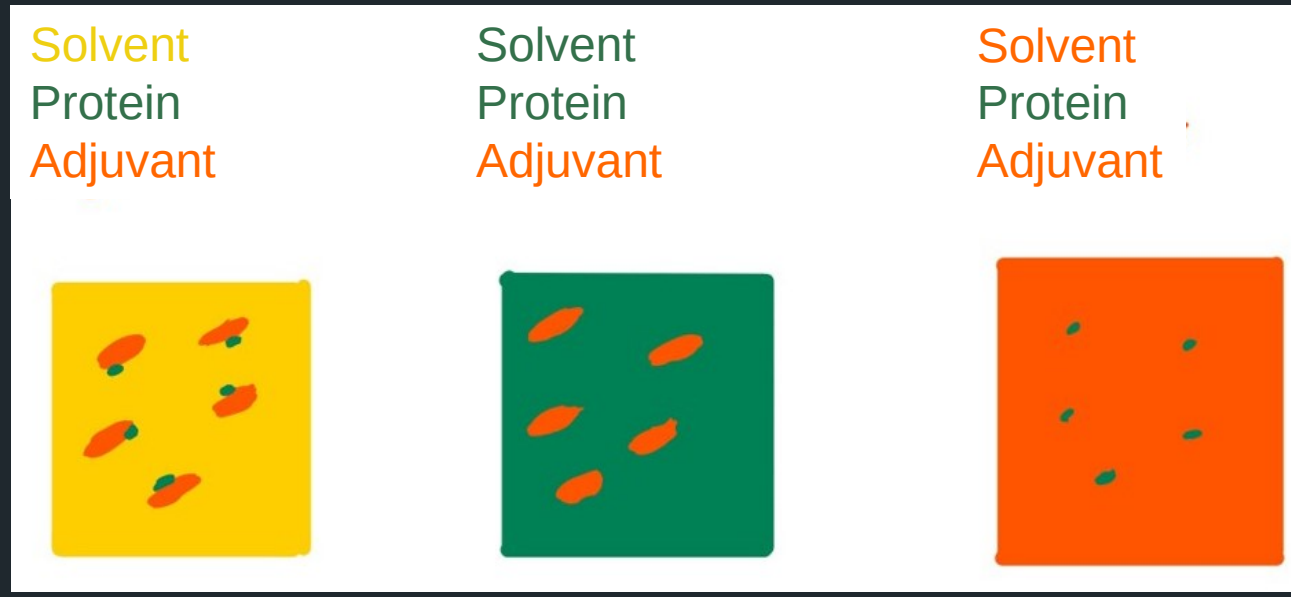
- Importance of Understanding the Effects of Freeze Thaw on Microstructures
  - Adjuvants
  - Proteins
  - Complexes
- Experiment

Effects of Freeze Thaw Cycles on Microstructures: SANS and Contrast Matching



Small Angle Neutron Scattering

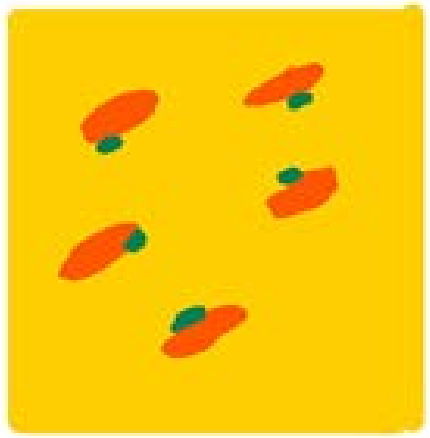
Contrast Matching

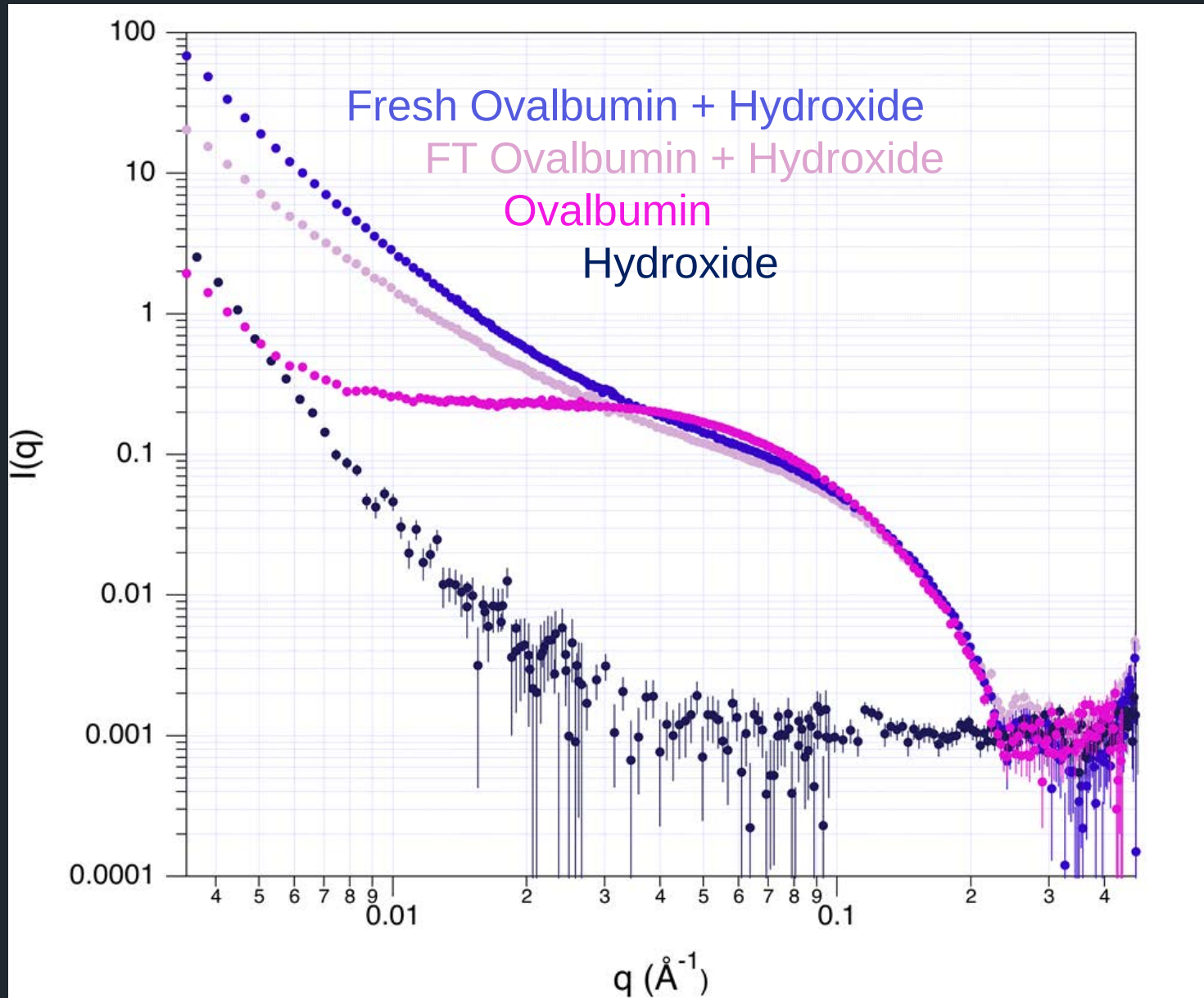


https://www.sciencedaily.com/releases/2016/11/161115094633.htm

Effects of Freeze Thaw Cycles on Microstructures: Small Angle Neutron Scattering

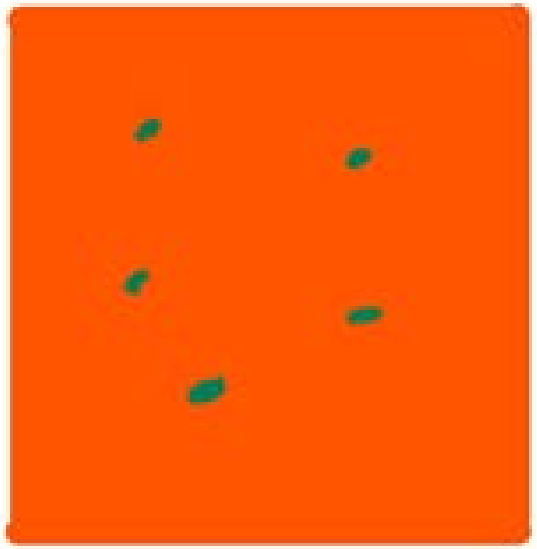
Solvent
Protein
Adjuvant

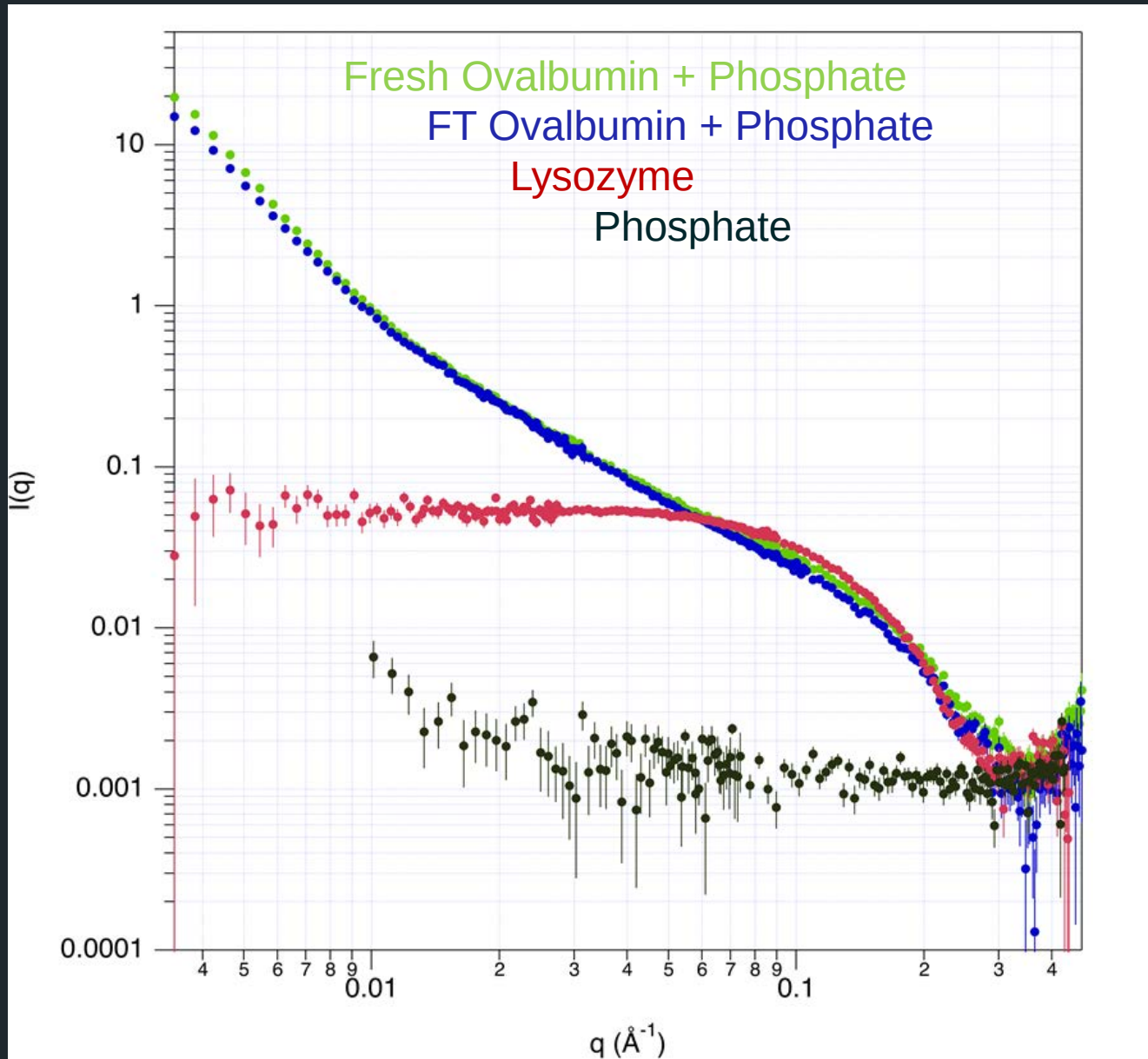




Effects of Freeze Thaw Cycles on Microstructures: Small Angle Neutron Scattering

Solvent
Protein
Adjuvant





Summary

- Interactions between proteins and adjuvants
  - Electrostatic interactions
  - Ligand exchange
- Effects of freeze thaw cycles on adjuvants and their interactions
  - Aggregation Hydroxide > Phosphate
  - More aggregation= less binding
- Effects of freeze thaw on microstructures of proteins, adjuvants, and complexes
  - Complexes form new structures
  - Protein 3D conformation is Maintained
  - Little change in adjuvant characteristics after freeze-thaw

Future Research/Acknowledgements

- Binding Isotherms in Sodium Chloride buffer
- Differential Scanning Calorimetry

- Dr. Amy Xu
- NIST/NCNR
- CHRNS
- SURF Directors
- Dr. Kathryn Sarachan
- Wilson College

Experimental Conditions

	Ovalbumin	Lysozyme	$Al(OH)_3$	$AlPO_4$
Isoelectric point	4.5	11.3	~11	4 – 5.5