Visualizing Insulin Structure Under Shear Stress

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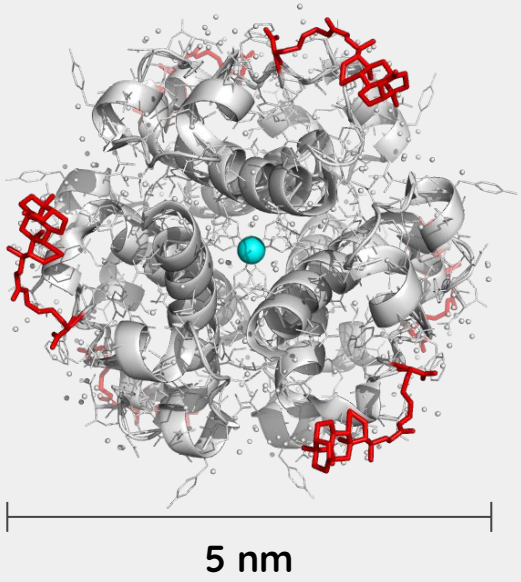


NIST
National Institute of Standards and Technology
U.S. Department of Commerce

NIST Center for Neutron Research

CHRNS
Center for High Resolution Neutron Scattering
NSF
NIST

Insulin is a hormone that **regulates glucose intake** by cells.

Patients with diabetes are **insulin-deficient**.

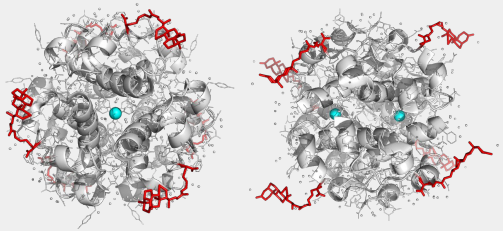
Diabetes is treated by injection of **engineered insulin analogues**.

INSULIN THERAPEUTICS

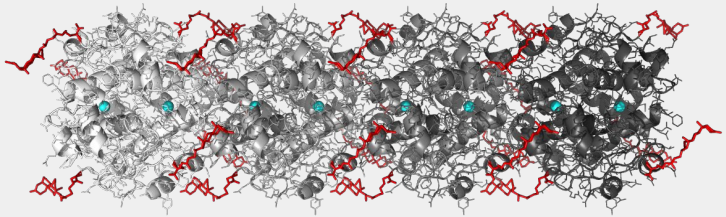
OBJECTIVE

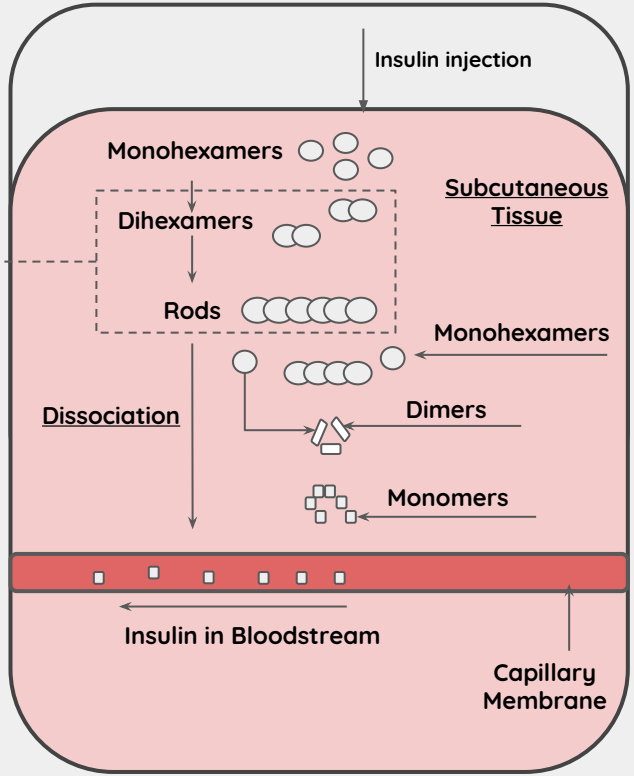
Long-acting insulin drug for once-daily dosing

Hexamers

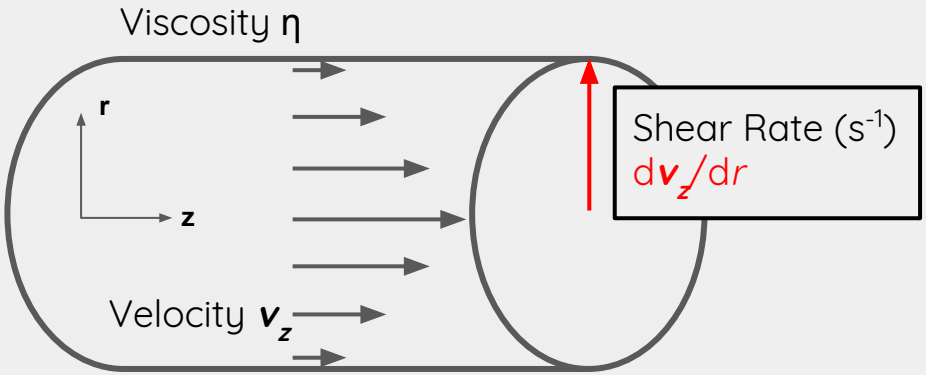


Rod-like Structures





Next Step: Does Shear Change Insulin Structure?



Subcutaneous Injection

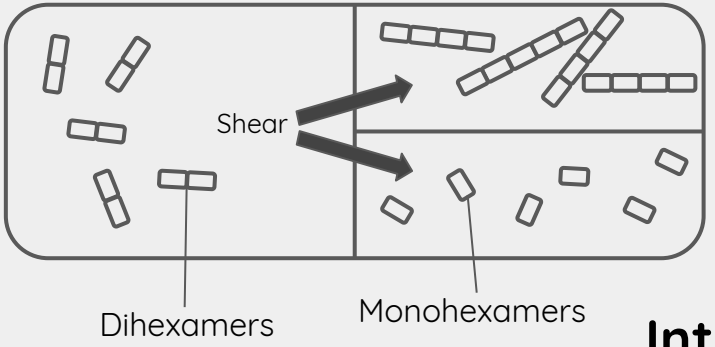
Shear rates on order of 10^5-10^6 s^{-1}

Blood Vessels

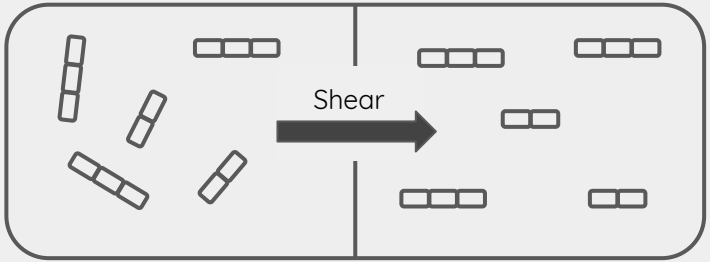
Shear stresses as high as 9.8 N/m^2

Possible Outcomes

Rod Lengthening/Breaking

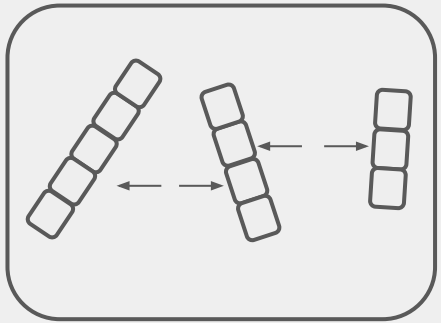


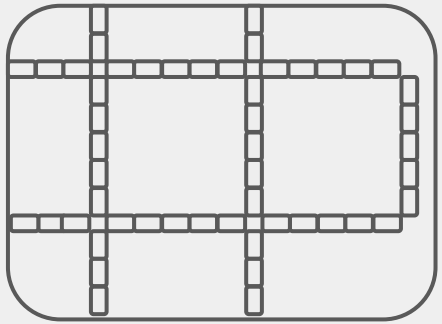
Alignment with Applied Shear



Interactions

Repulsion





Attraction

Project Overview

Objective: Determine if shear affects insulin self-assembly

♦

Insulin samples prepared:

Insulin concentrations (mM): **0.6** | 1.2 | 1.8

NaCl concentrations (mM): **30** | **150** | 450

Formulation

Physiological

Screens electrostatic repulsions

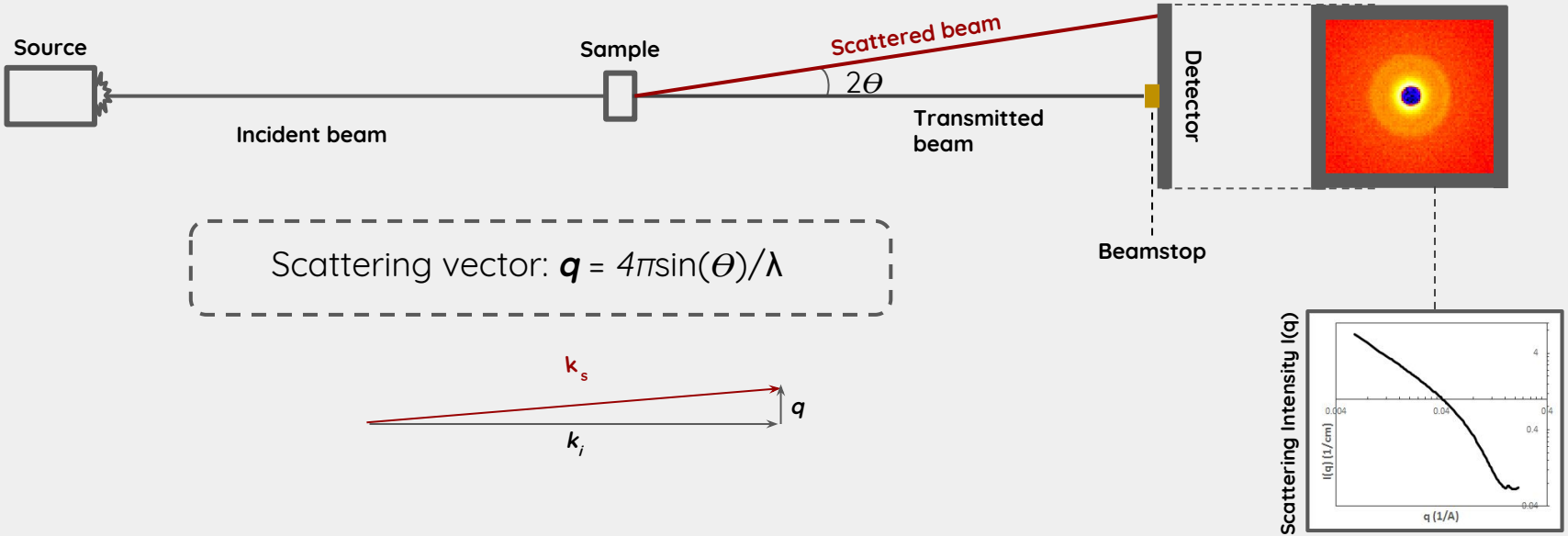
♦

Samples sheared at different shear rates to probe their **viscosity**

♦

Small-angle neutron scattering applied during shear for structural data

Viewing Nanostructure With Small Angle Neutron Scattering

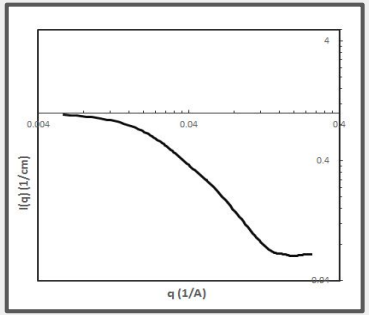


Why SANS?

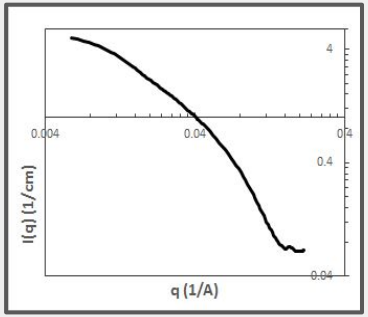
Probes appropriate length scale to view:

- Structure
- Orientation of structures

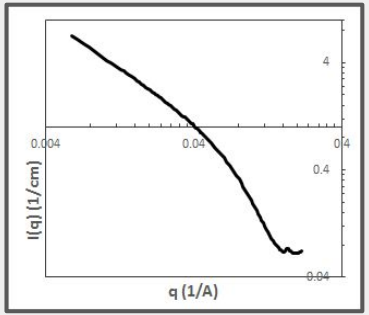
5 Hexamers per rod



10 Hexamers per rod



20 Hexamers per rod



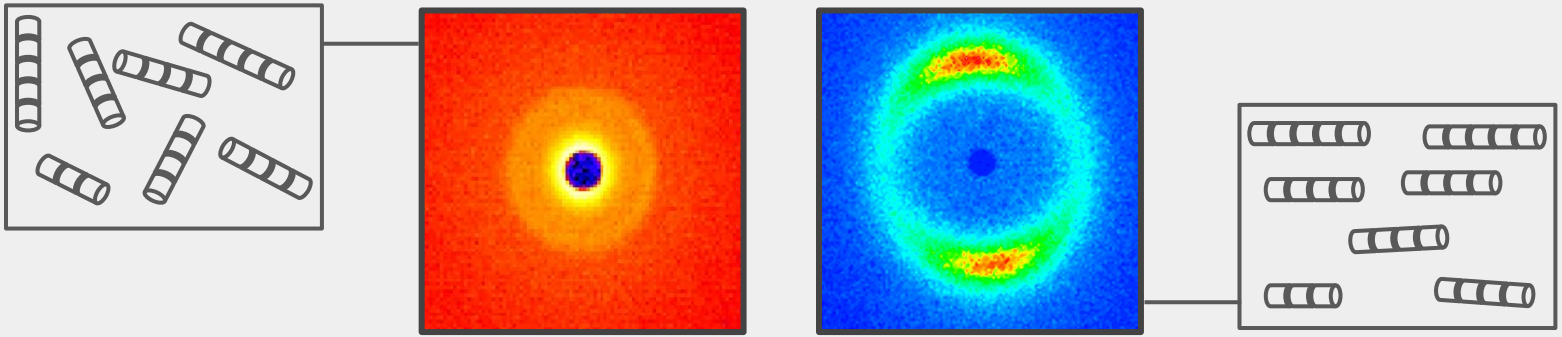


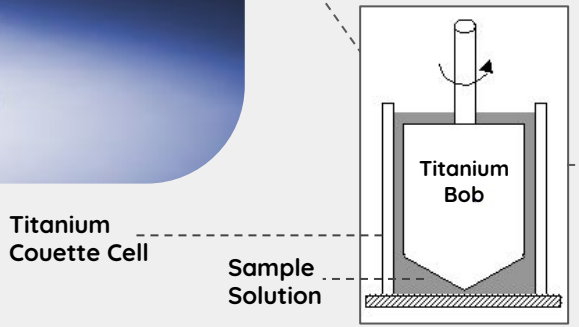
Image from http://cns.che.udel.edu/

Rheology Measurements

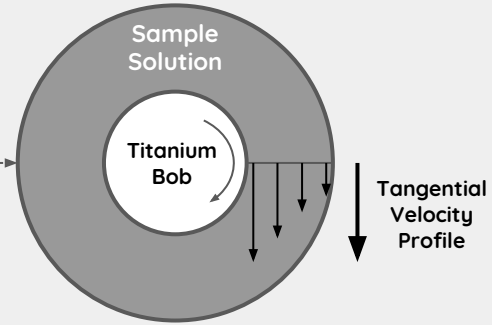
Images:
Rheometer:
http://www.anton-paar.com/ca-en/products/details/rheome
Cup and Bob:
ter-mcr-102-302-502/
https://ciks.cbt.nist.gov/~garbocz/SP946/node14.htm

Instrument: Anton Paar MCR 502 Rheometer

Shear Rates: 0-3500 s^{-1}

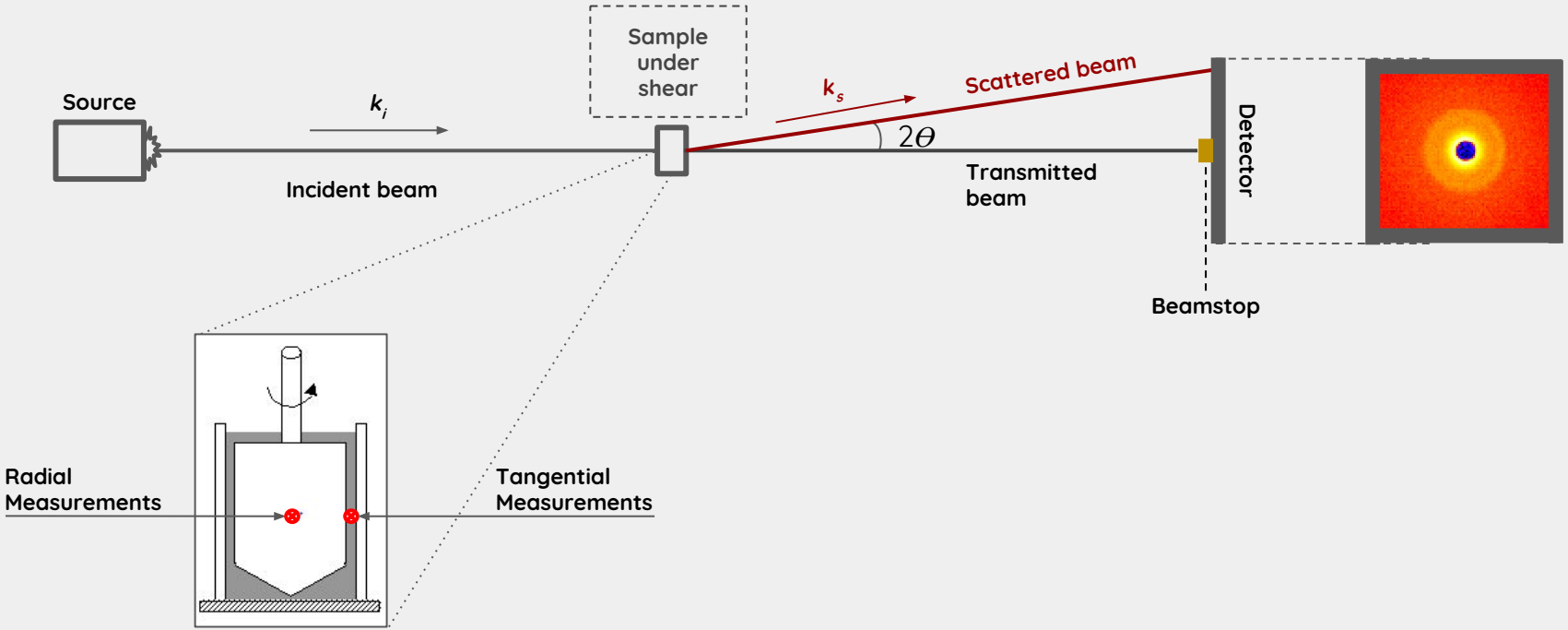


Front View

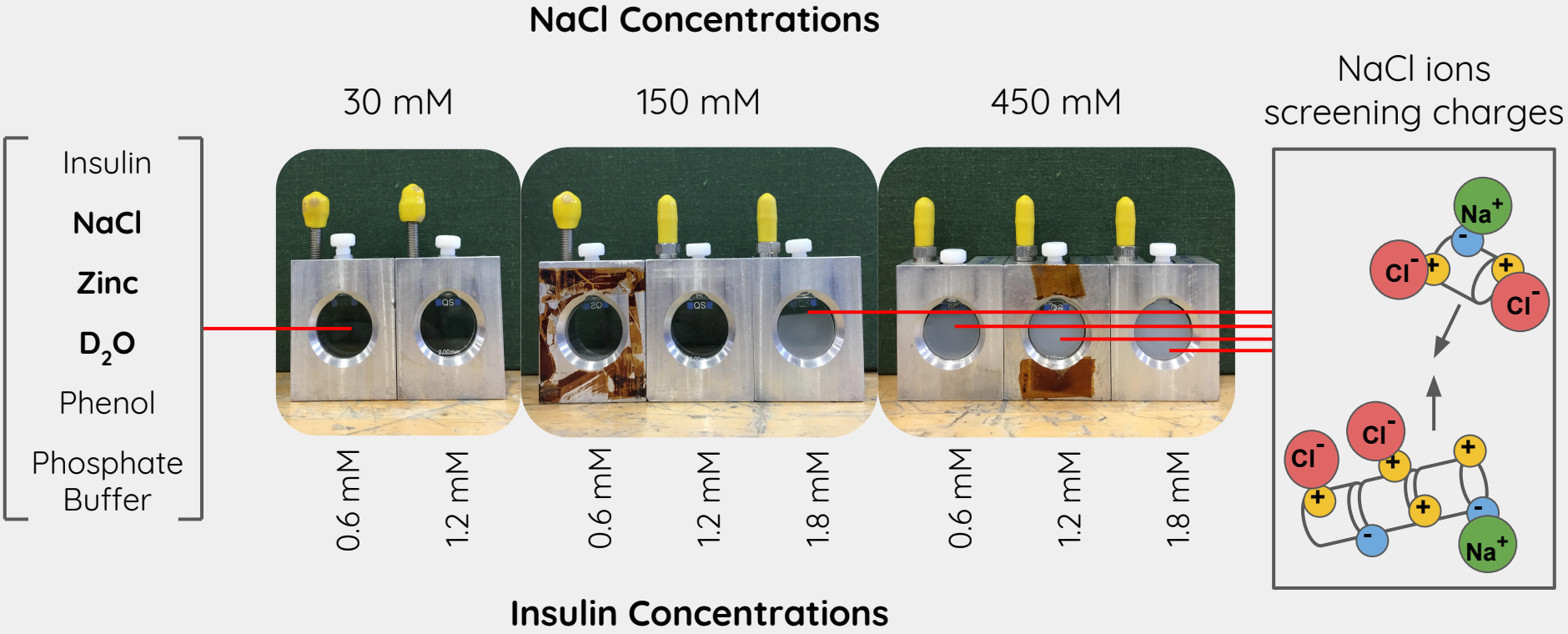


Top View

SANS Measurement

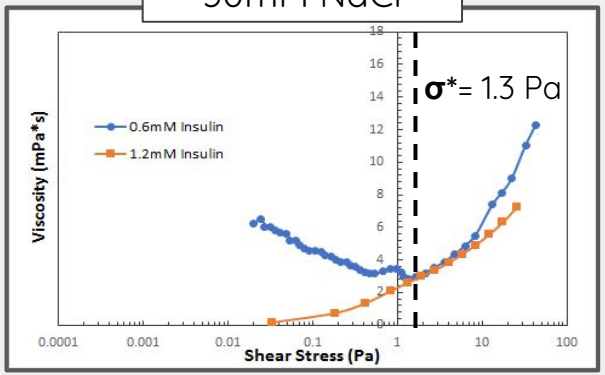


Sample Preparation

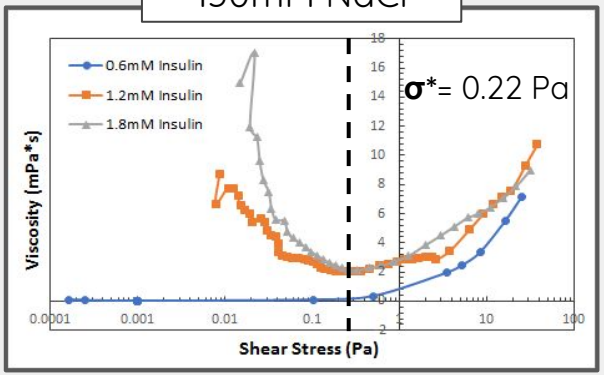


Rheology: Viscosity vs. Shear Stress

30mM NaCl

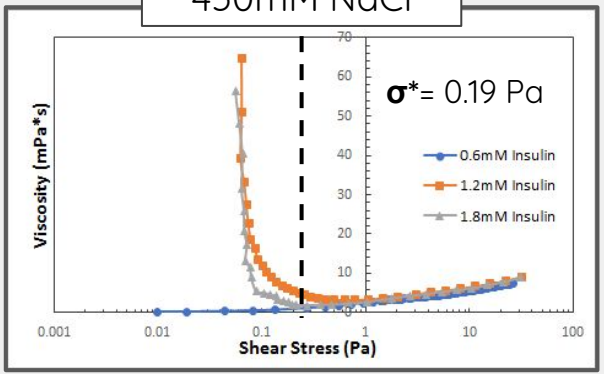


150mM NaCl

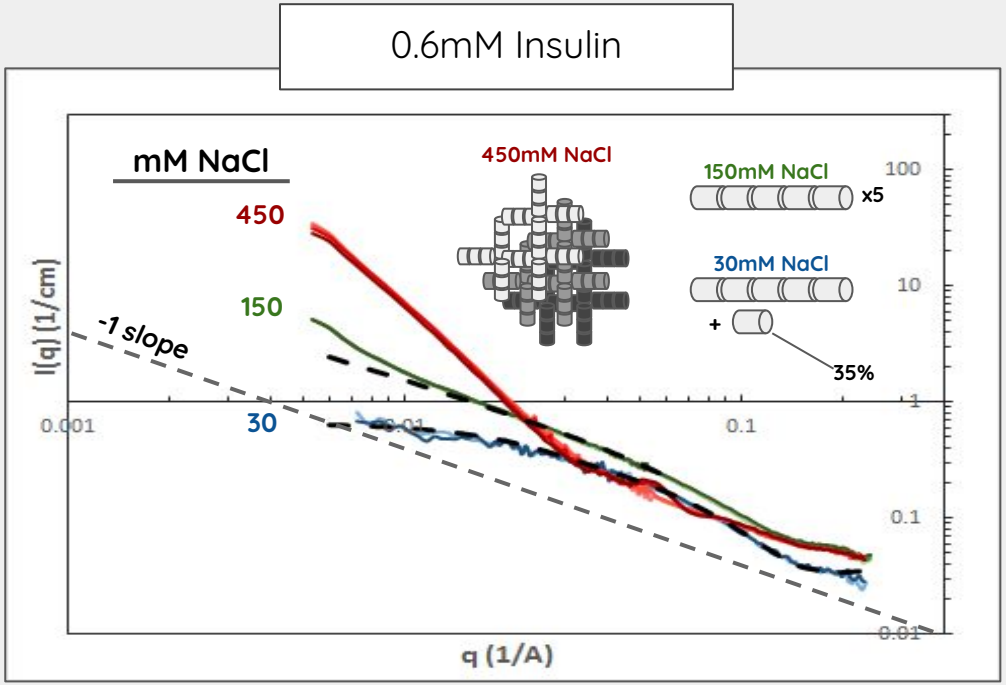


Insulin Concentration	Volume Percentage
0.6mM	0.44%
1.2mM	0.88%
1.8mM	1.32%

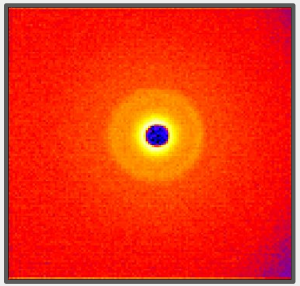
450mM NaCl



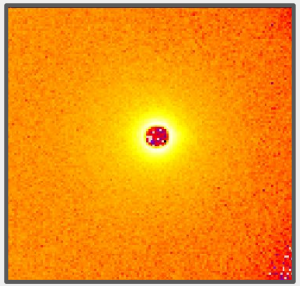
SANS Data: Increasing Salt Concentration



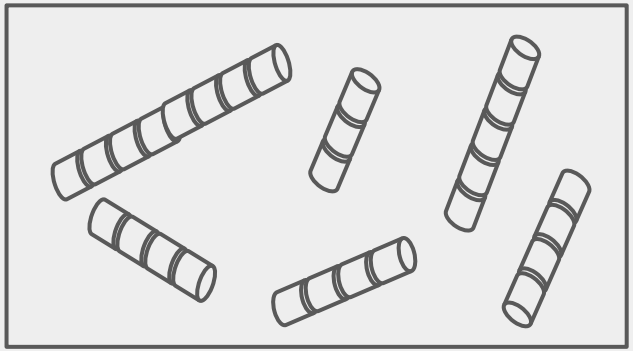
Radial Configuration



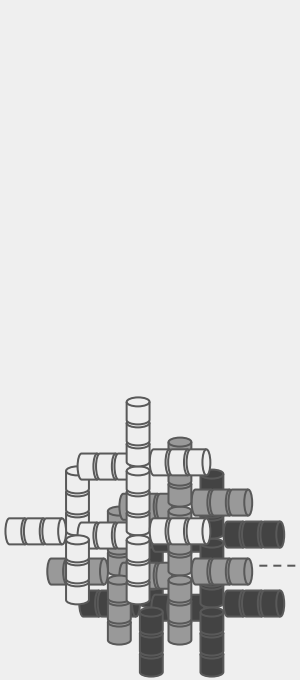
Tangential Configuration

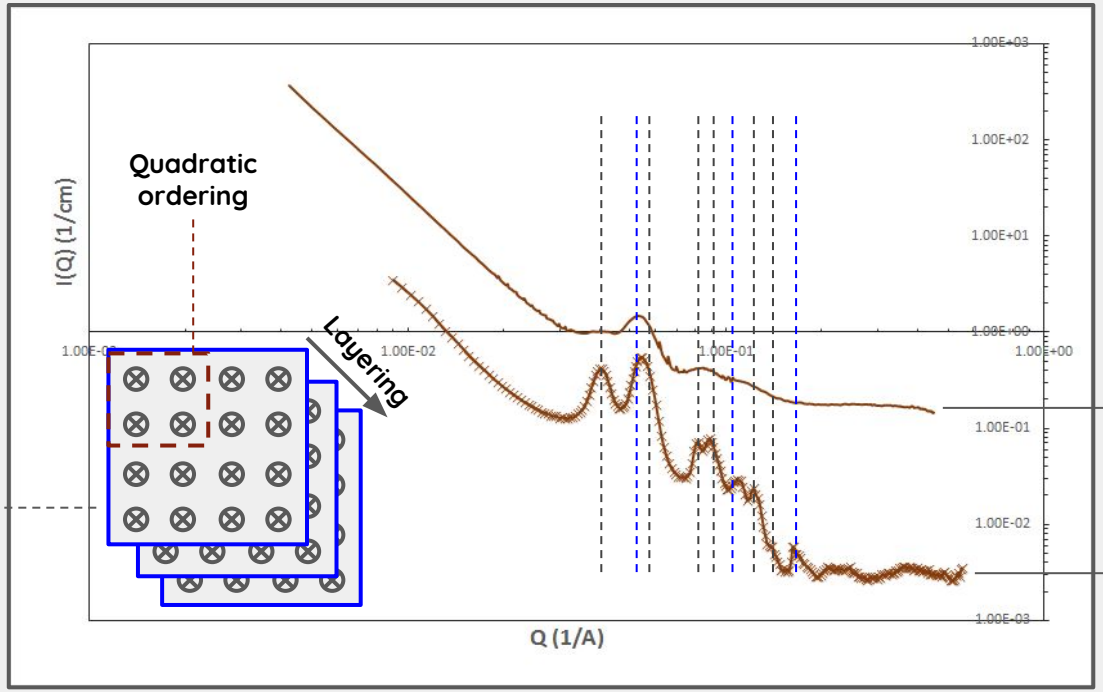


Isotropic Orientation



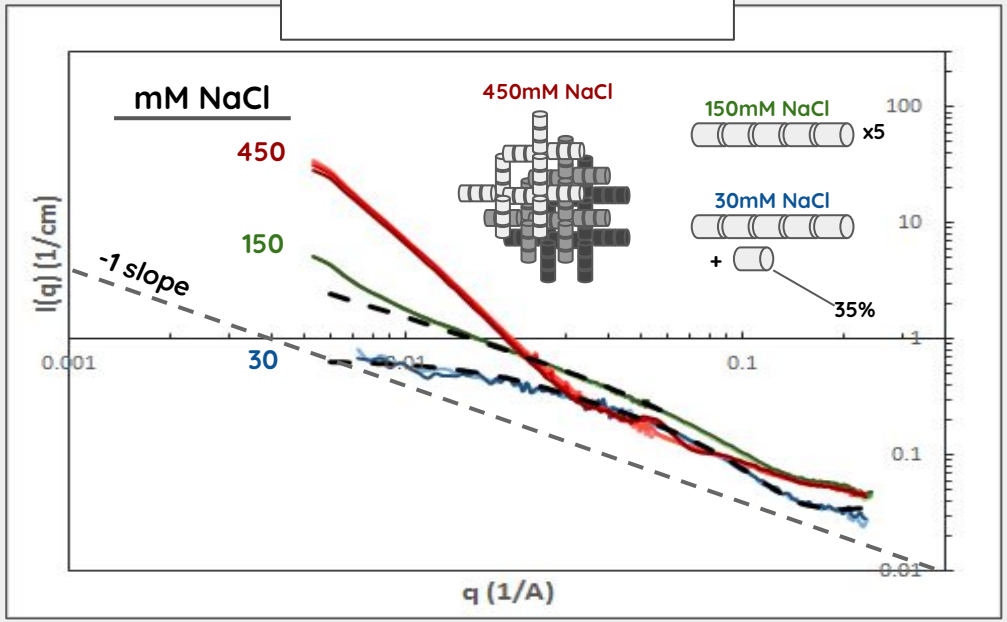
Higher Order Structures: SAXS Comparison



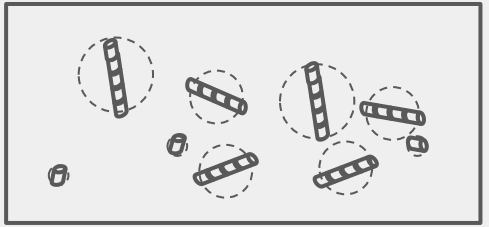


Shear Thickening Dependence on Shape

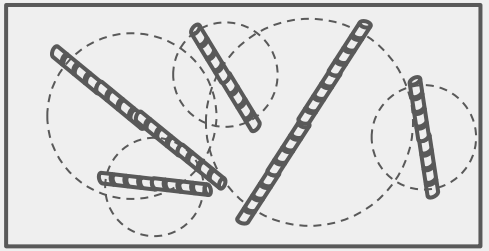
0.6mM Insulin



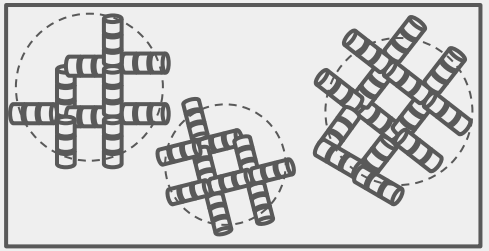
30mM NaCl



150mM NaCl



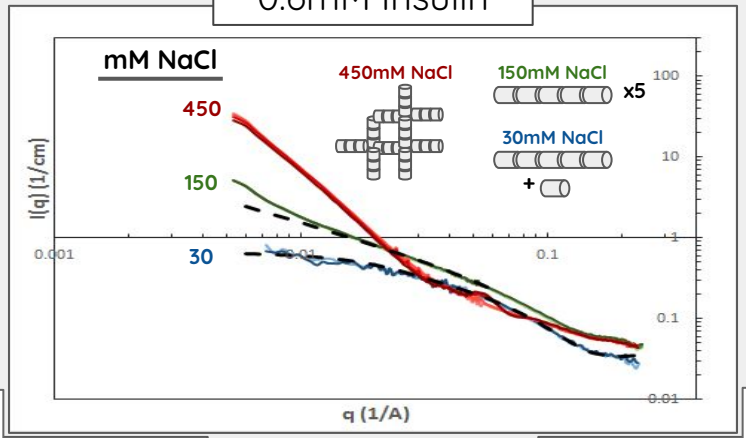
450mM NaCl



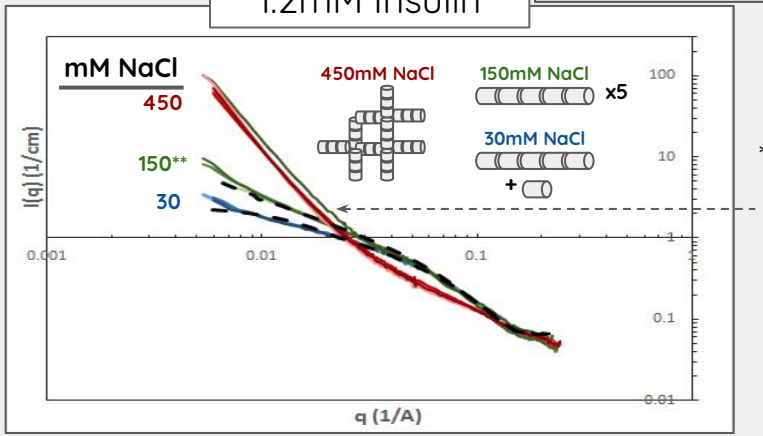
SANS Data: Increasing Salt Concentration

– – – – Fitted models

0.6mM Insulin

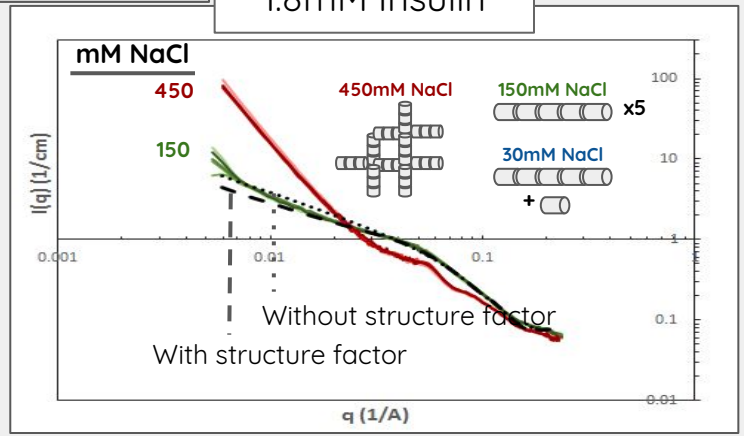


1.2mM Insulin

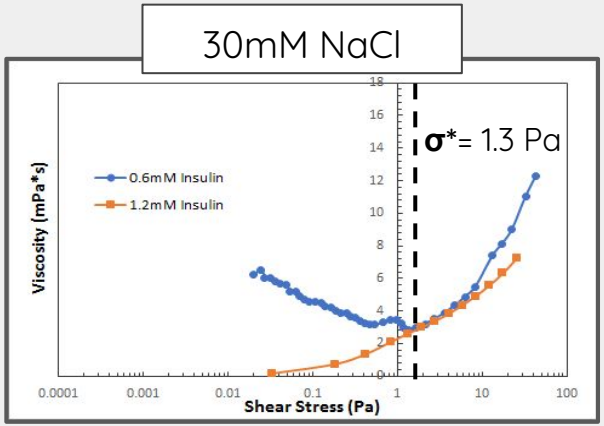


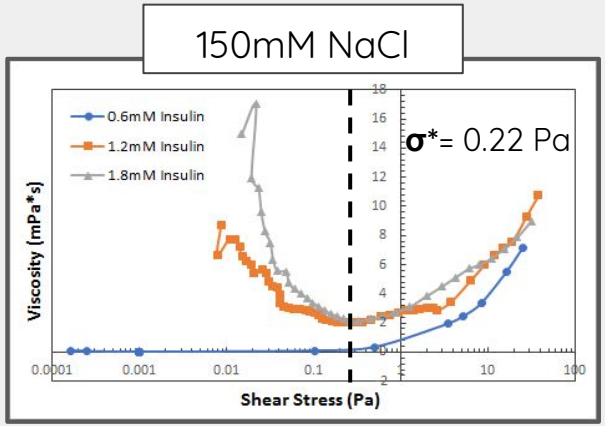
Shear-induced reversible structural change at **150mM NaCl, 3500s^{-1}

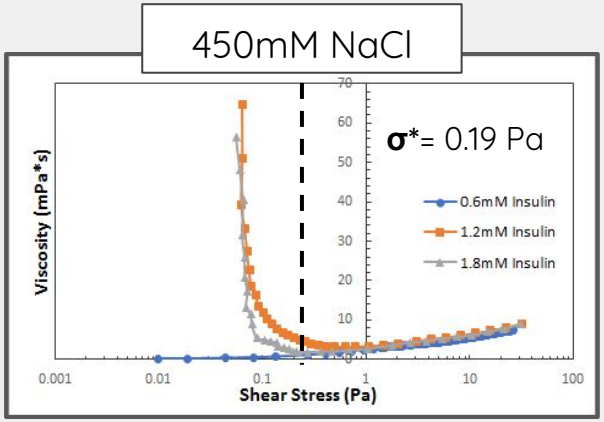
1.8mM Insulin

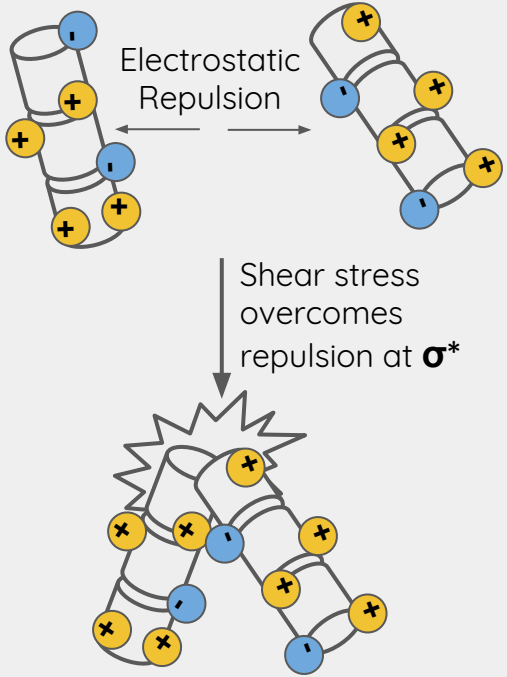


Electrostatic Interactions?





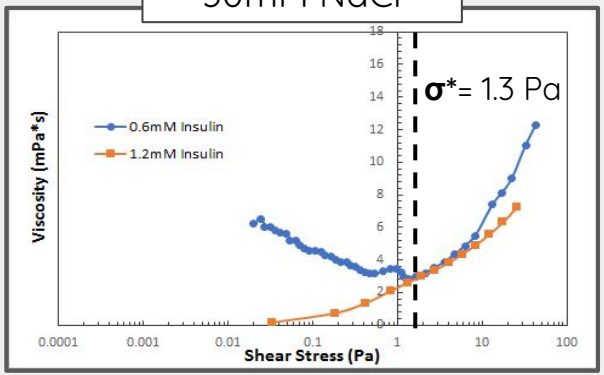




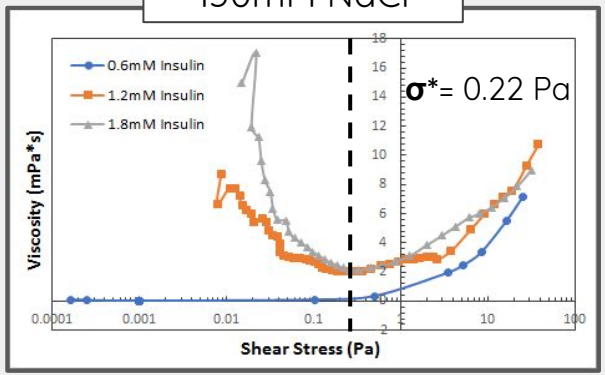
SHEAR THICKENING

Electrostatic Interactions?

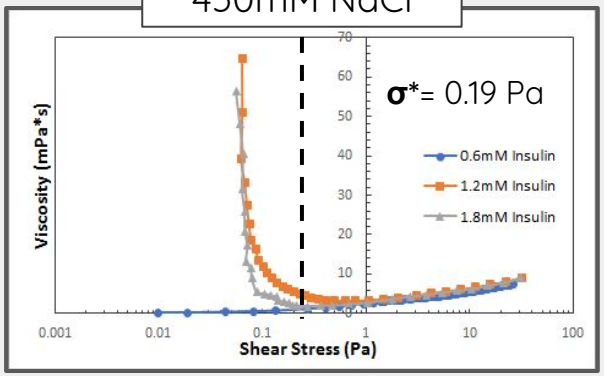
30mM NaCl



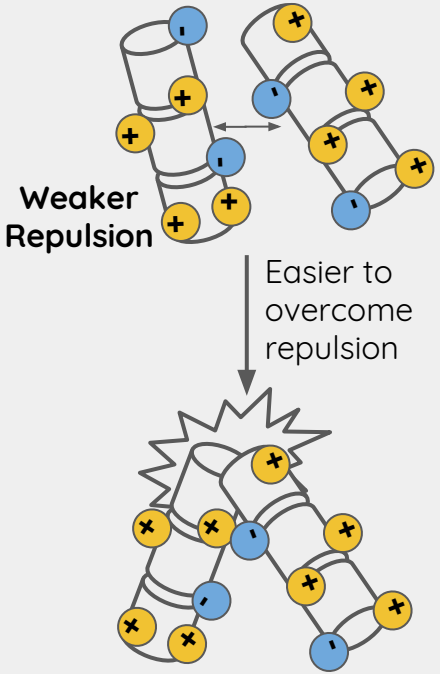
150mM NaCl



450mM NaCl



With NaCl Screening



Higher NaCl = Lower σ*

Summary of Findings

Structure

Rods **form, lengthen, and order** with increasing NaCl and insulin concentrations

Very little shear dependence

Rheology

Shear-thinning ⟶ Shear-thickening

Critical shear stress (electrostatic barrier?)

Future Outlook

Tests with higher shear rates, lower q

More rheological studies

Acknowledgments

Center for High Resolution Neutron Scattering

Novo Nordisk

Grethe Vestergaard Jensen, PhD

Mentor, NIST Center for Neutron Research

Joe Dura, PhD and Julie Borchers, PhD

NCNR SURF Coordinators, NIST Center for Neutron Research

Brandi Toliver, PhD

SURF Program Director, National Institute of Standards and Technology

NIST
National Institute of Standards and Technology
U.S. Department of Commerce

NIST Center for Neutron Research

NSF CHRNS Center for High Resolution Neutron Scattering NIST