D2O AND THE LIPIDIC CUBIC PHASE

Stacy Young
Mentor: Tom Cleveland

INTRODUCTION

2 components to project:

- Protein preparation + crystallization
- Determining the phase diagram of monoolein in D2O

BACKGROUND

Why do we crystallize proteins?

- Analysis via xray scattering

  →structural information revealed

2 requirements of crystallization:

1) Protein stability
2) Diffusible environment

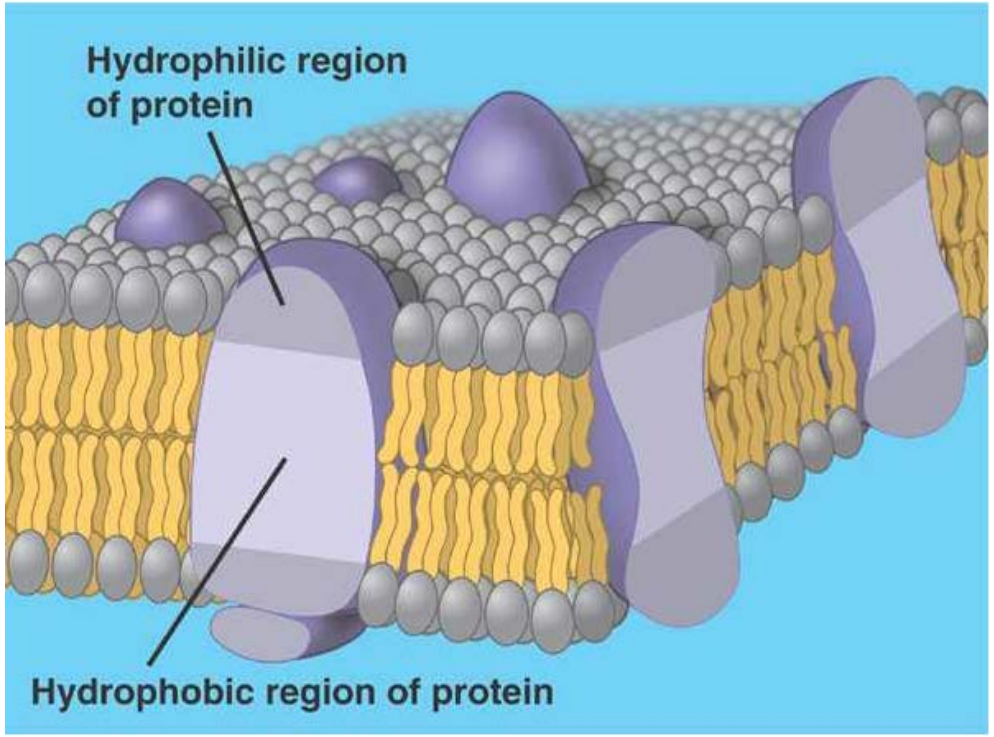
2 types of proteins

- Soluble vs. membrane

BACKGROUND

Membrane proteins:

- Embedded within lipid bilayer
- Lose structural + functional stability when removed from membrane
- Critical role in cell processes
- 26+% of proteins coded by human genome = membrane proteins*
- Over 50% of drugs target membrane proteins+
- As of 2014, 406 out 36,000 identified proteins are membrane proteins*



Source: http://goo.gl/kAFA0C

*Source: 2014 - Kynde et al. - Small-angle scattering gives direct structural information about a membrane protein inside a lipid environment.pdf

+http://www.irbbarcelona.org/en/news/more-than-50-of-drugs-target-membrane-proteins

BACKGROUND

Problems with methodology

- Protein isolated, mixed with buffers + precipitants
- Mostly only works with soluble proteins
- Membrane proteins ….?
  - Not in solution
  - Need to be purified but protected at the same time

METHODS: BR

Bacteriorhodopsin (bR) isolated from *Halobacteria*

1) Grow organism
2) Harvest
2) Lyse
3) Isolate membranes
4) Solubilize with octyl glucoside (OG)

Image from experiment: Halobacteria culturing

BR EXPRESSION AND PURIFICATION

Cell Pellet

Low-Salt Lysis

Washed Membranes

Sucrose Gradient

"Purple Membrane"

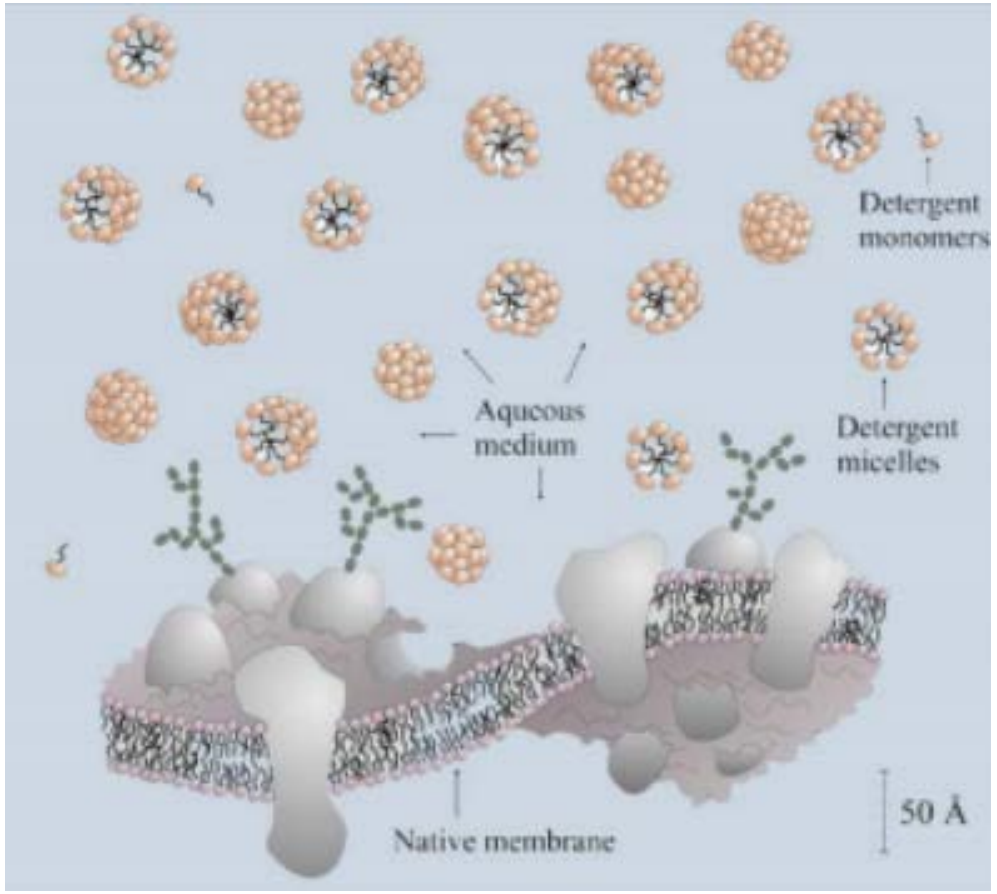
METHODS: BR

OG

- Detergent
- Solublizes protein, prevents large masses from aggregating
- Used to remove bR from membrane
- Formation of detergent micelles

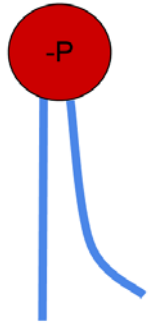
Protein purified…but still cannot crystallize

- Protein placed in lipidic cubic phase



Source: [Caf03] Caffrey, M. (2003) Membrane protein crystallization. Journal of Structural Biology ***142****:108-132.*

Standard phospholipid



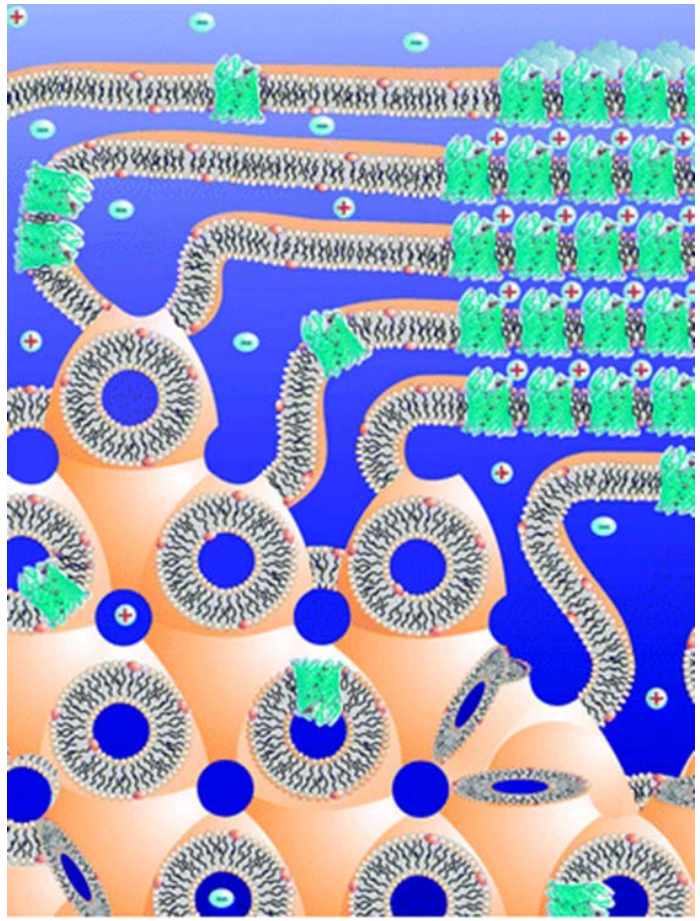
Source: http://goo.gl/KVfuEQ

LIPIDIC CUBIC PHASE

Special lipid environment

Satisfies conditions for crystallization

Variations of cubic phases



Source: http://goo.gl/4SUlus

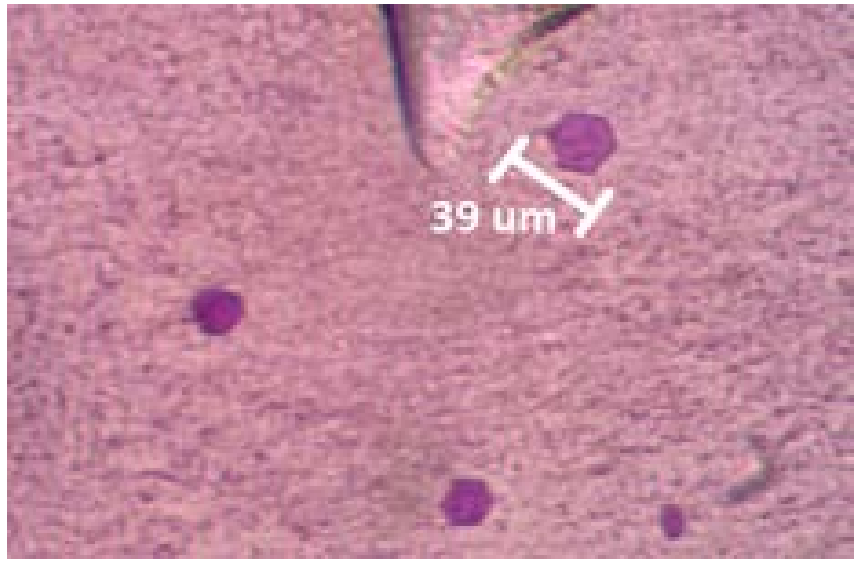
CRYSTALLIZED PROTEIN

Placed 0.2 µL of cubic phase containing protein on slide

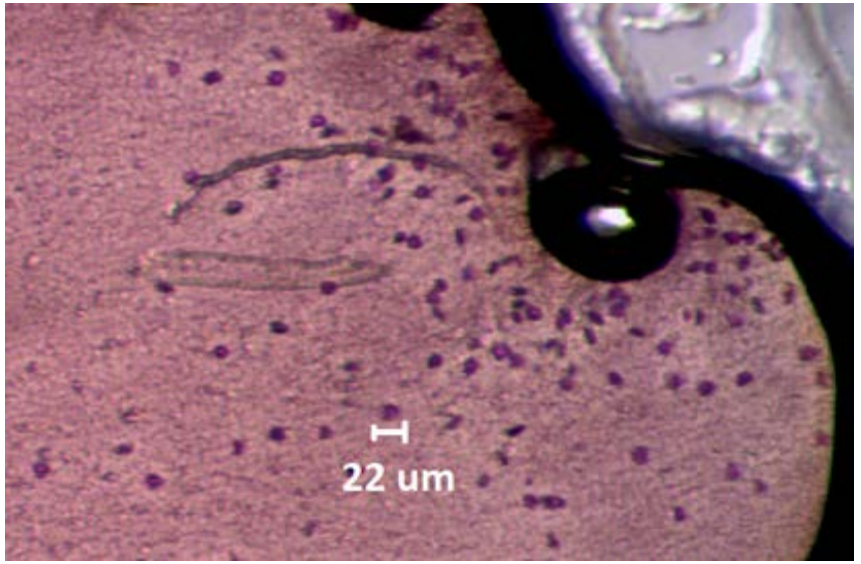
Layered 1 µL of phosphate precipitant onto each cubic phase drop

Crystals form over about 1.5 weeks

Crucial experimental checkpoint



1.8 M Na/K Phosphate pH 5.6



2.6 M Na/K Phosphate pH 5.6

EXPERIMENTAL OBJECTIVE

2 knowns:

- bR placed in lipidic cubic phase
- bR WILL crystallize
- How?

Small Angle Neutron Scattering (SANS)

- Examine early intermediates of protein crystal formation

METHODS: PHASE DIAGRAMS

Want to observe protein

contrast match out lipid

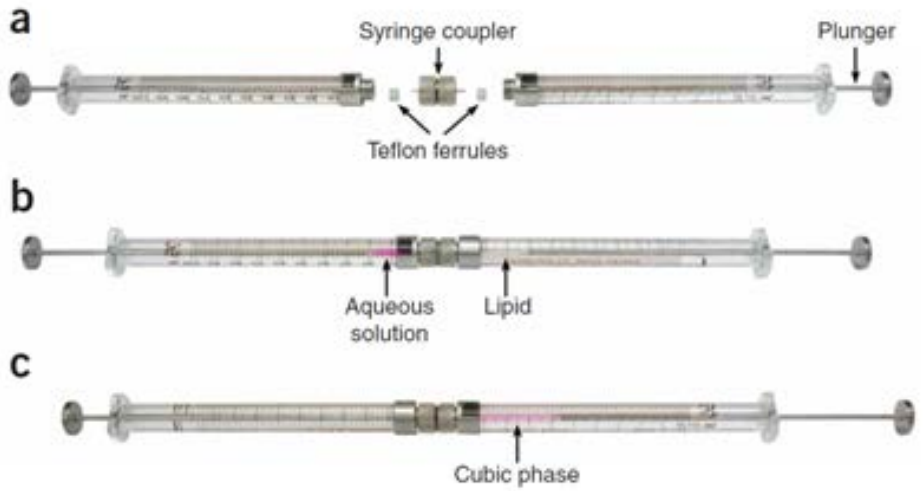
Creation of a phase diagram of monoolein in D2O

- determine which conditions yield which cubic phase
- Want to understand exactly where we are in the phase diagram

METHODS: PHASE DIAGRAMS

Capillaries

- filled with monoolein and varying concentrations of D2O or H2O

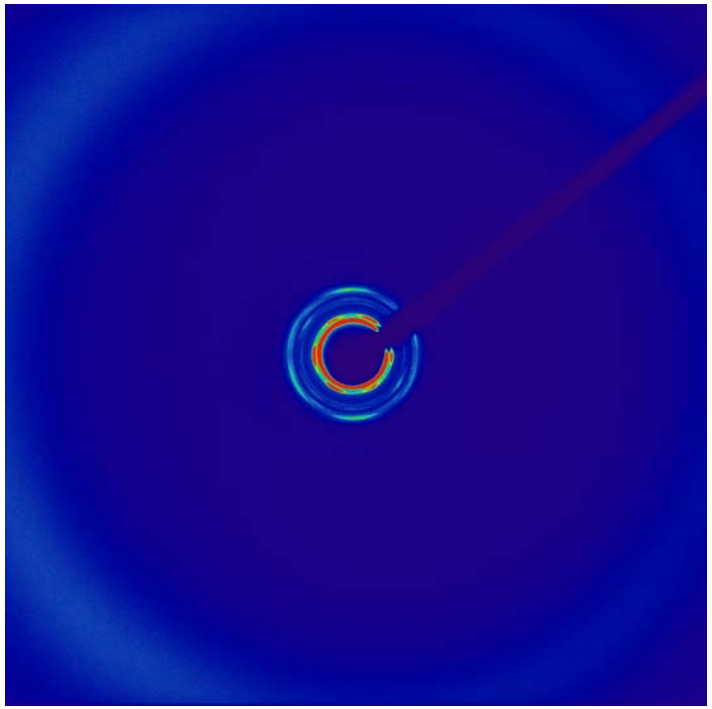


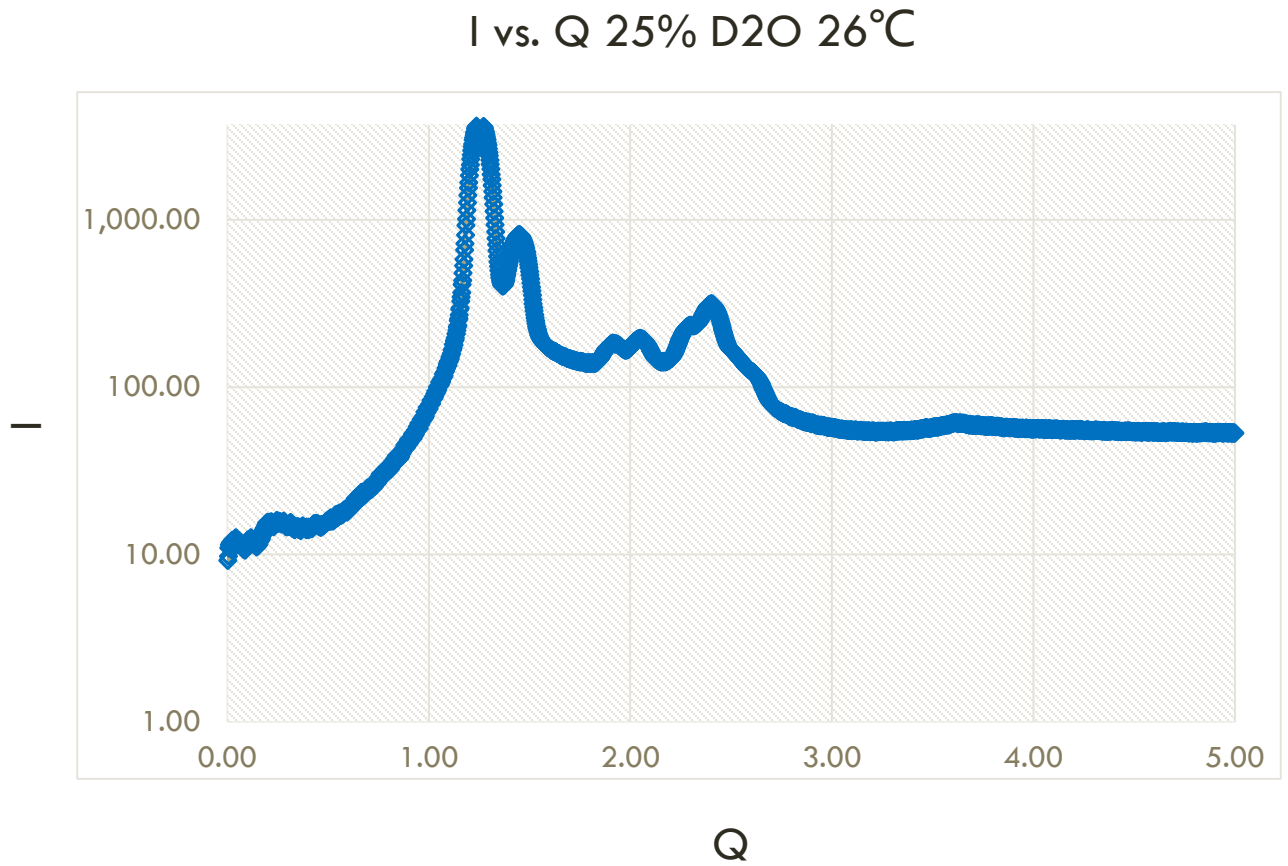
analyzed via Small Angle X-ray Scattering (SAXS) in different temperatures (17℃-30 ℃)

Fit2d program used to generate I vs. Q graph from scattering image

CAPILLARIES: SAXS IMAGE + CORRESPONDING GRAPH

25% D2O concentration



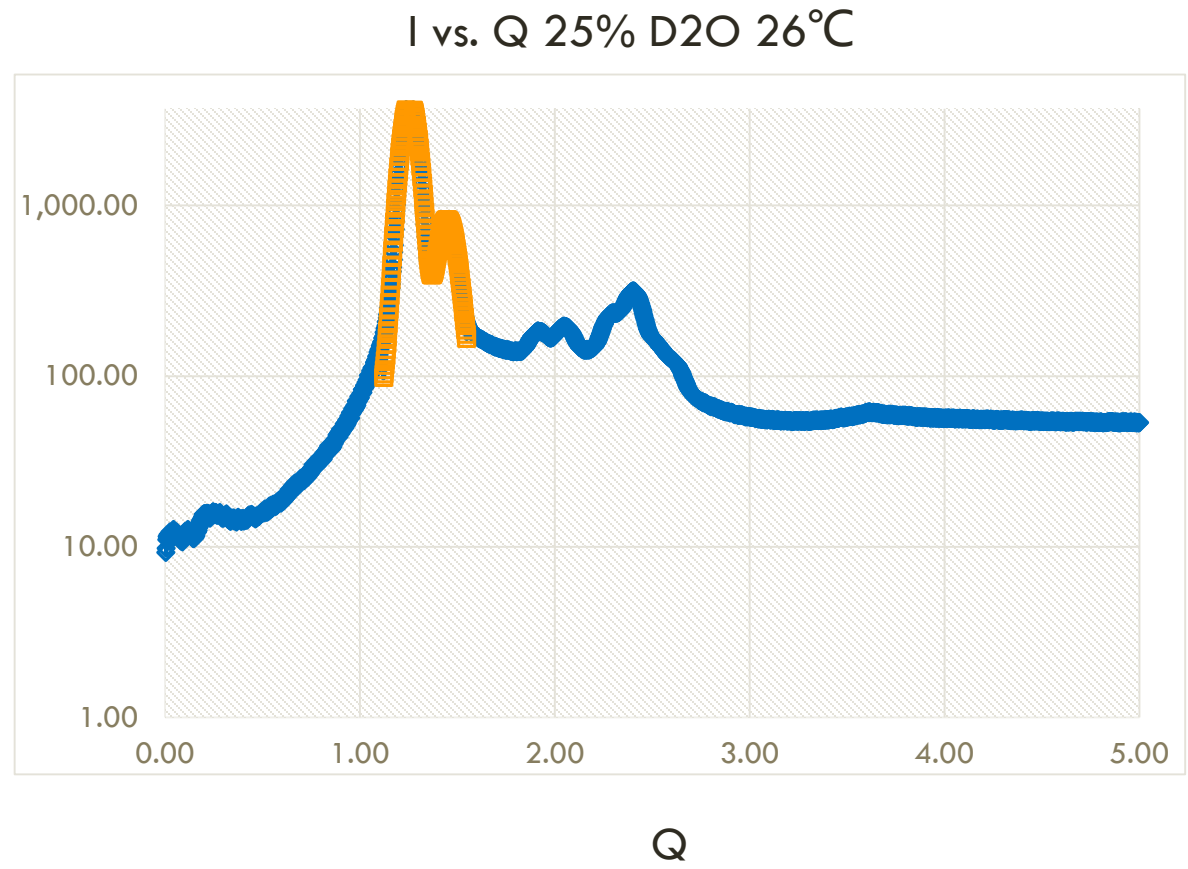


CAPILLARIES: SAXS IMAGE + FITTED GRAPH

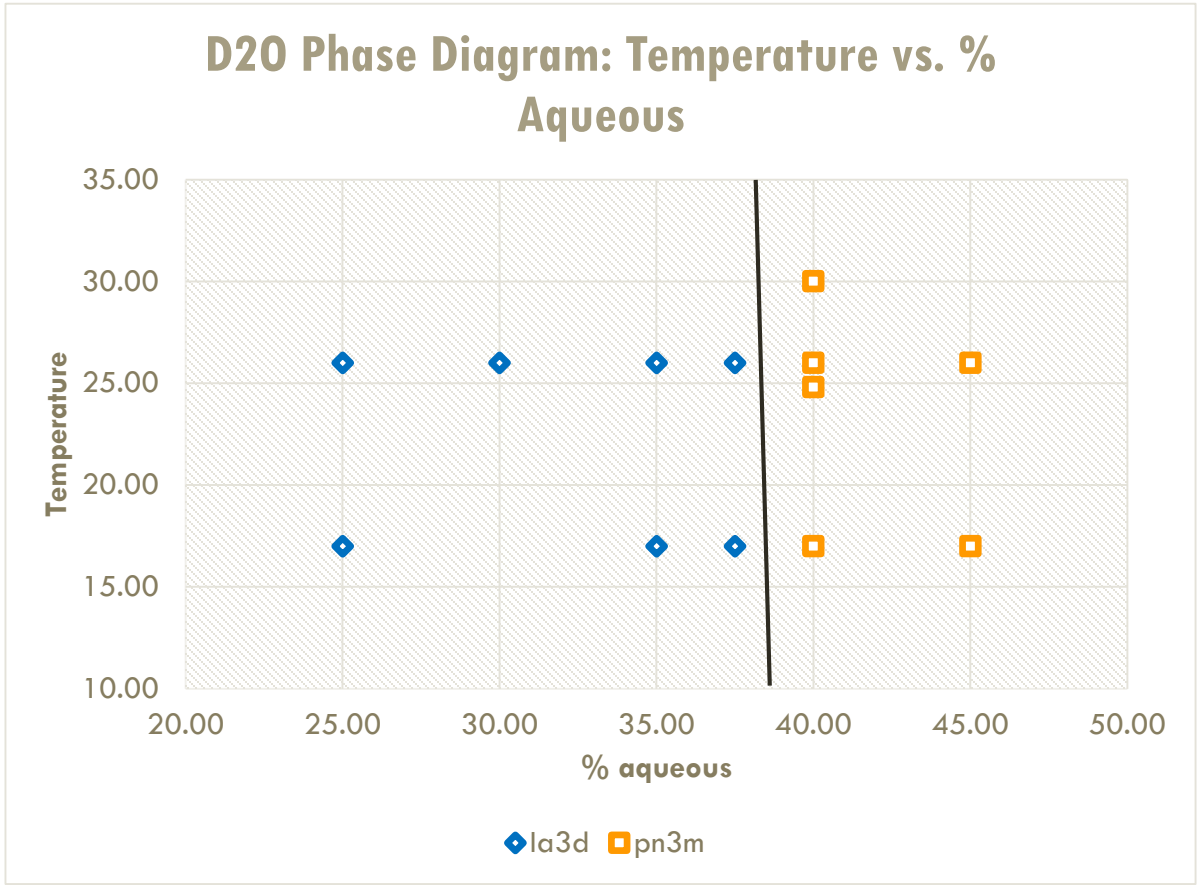
first two graph peaks fit using a Gaussian function

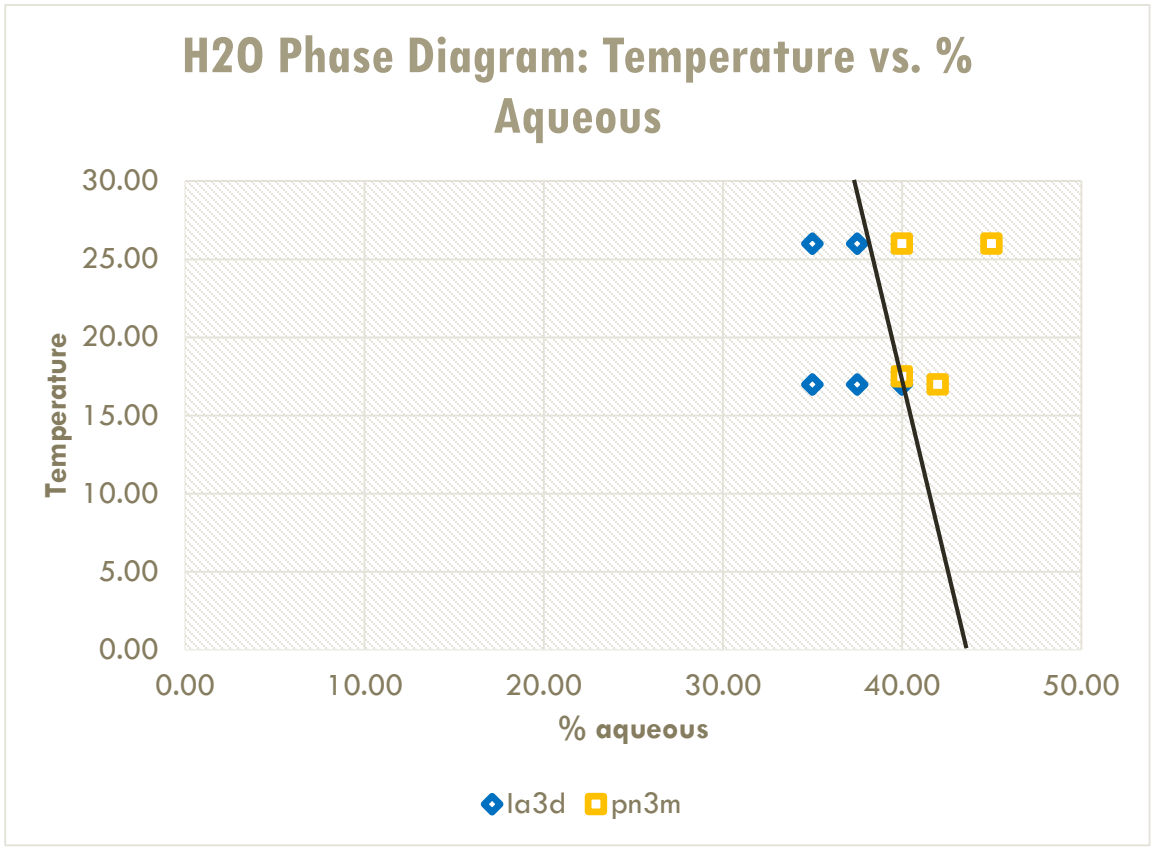
- determine position

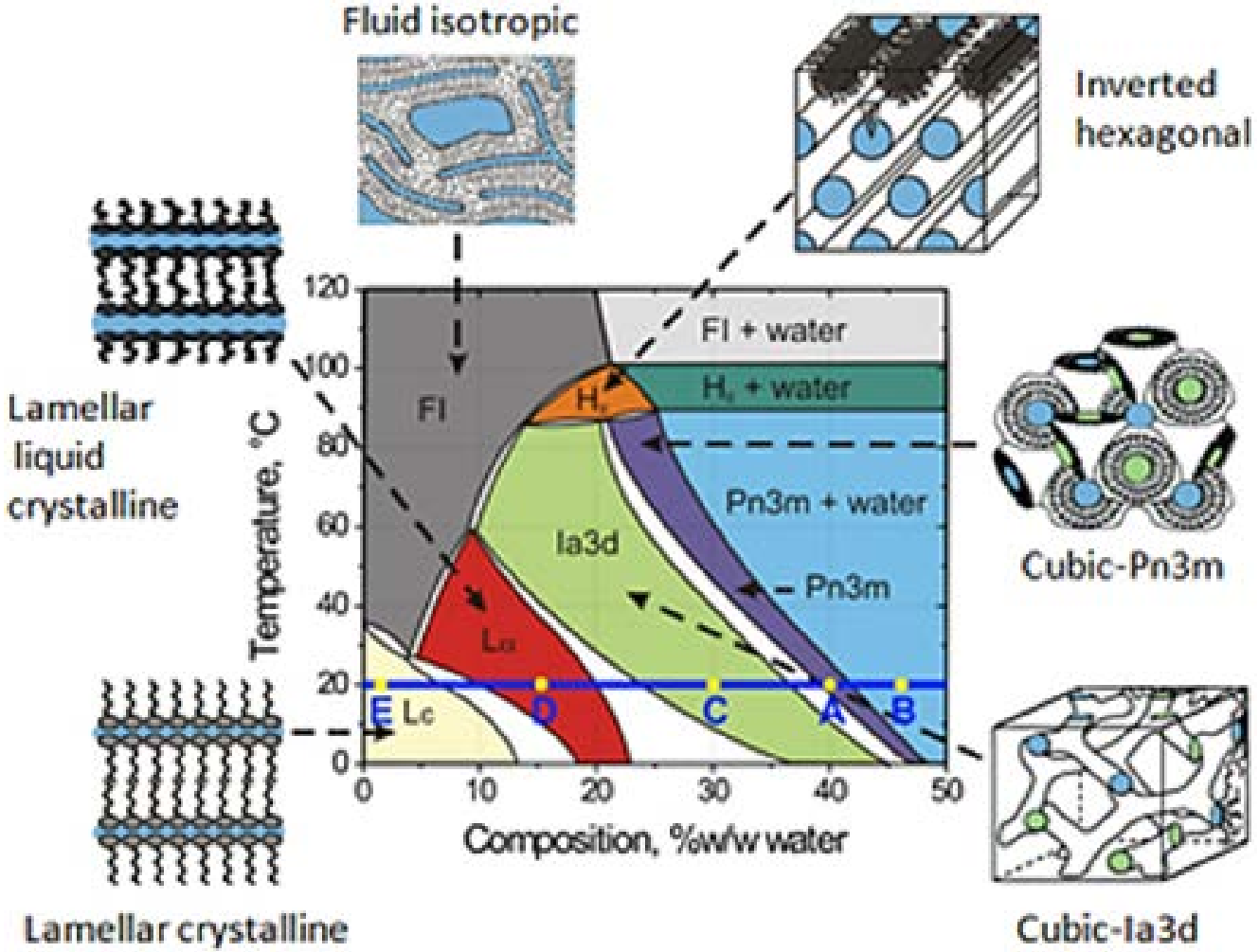
ratio of peak position indicated the given cubic phase



PHASE DIAGRAM



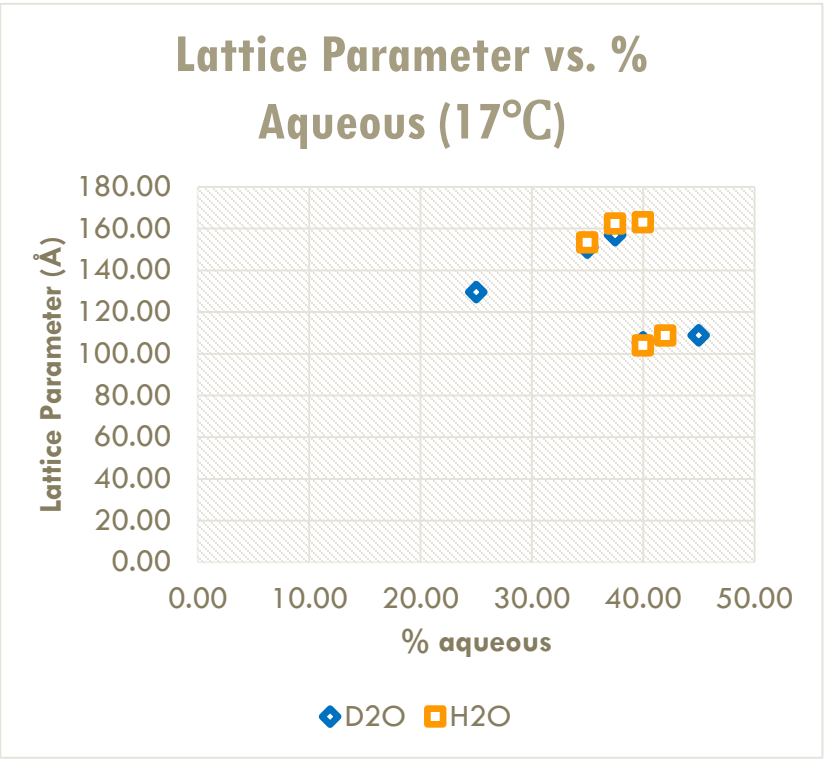


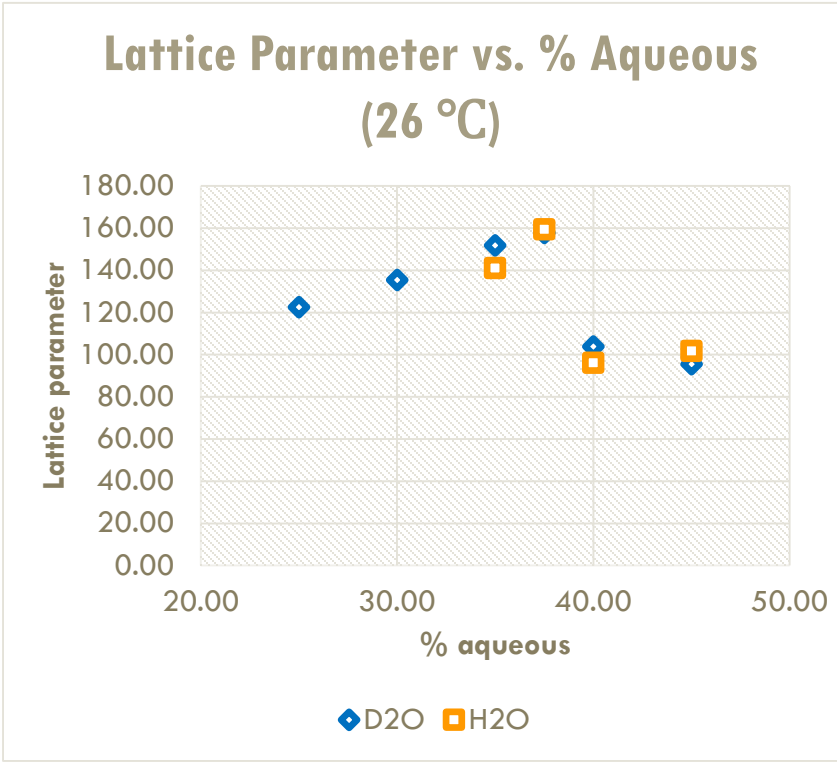


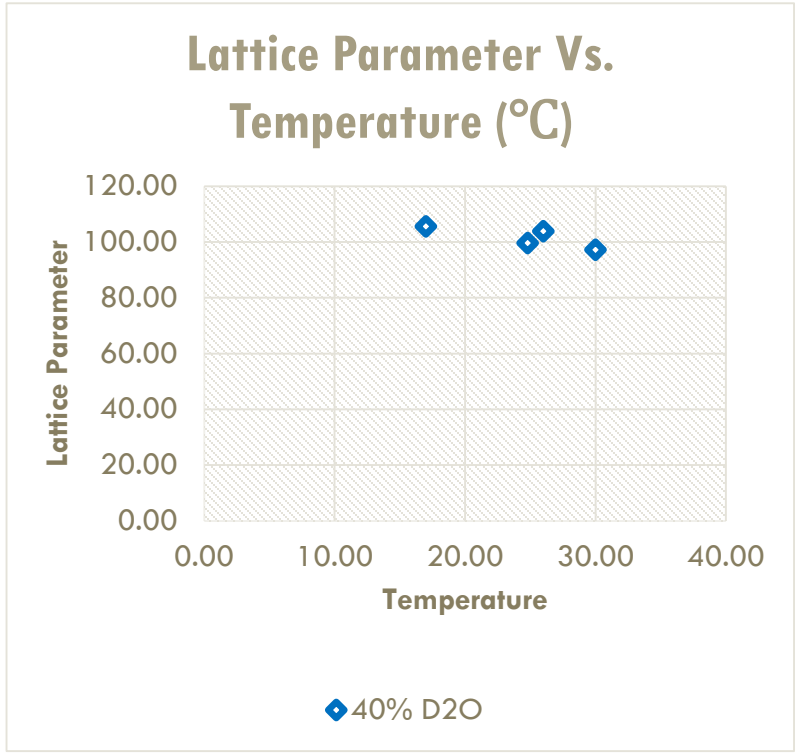
H2O Phase Diagram (from literature)

Source: http://cherezov.usc.edu/resources.htm

LATTICE PARAMETER VS. % AQUEOUS







BIG PICTURE…WHO CARES?

bR "easy" membrane protein, structure has already been identified

- Why spend so much time with this protein?

Advantageous to study exact process of protein crystal formation

- bR excellent sample group
- Process applied to more difficult membrane proteins

Pharmaceutical applications

- More advanced drugs

FINAL THOUGHTS

Structure, Structure, Structure!

- Entirety of life dependent on the interaction and workings of proteins

Small step…but large stride

ACKNOWLEDGEMENTS

Dr. Thomas Cleveland

Dr. Zvi Kellman

Dr. Paul Butler

Dr. Julie Borchers & Dr. Yamali Hernandez

NIST SHIP Director

NIST Center for Neutron Research

Institute for Bioscience and Biotechnology Research

National Science Foundation + CHRNS