Image from https://www.pinterest.com/explore/funny-cows/

# Small angle scattering study of NIST SRM1549a: **Bovine Whole Milk Powder**

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Summer Undergraduate Research Fellowship
at the NIST Center for Neutron Research

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

NSF CHRNS CENTER FOR HIGH RESOLUTION NEUTRON SCATTERING NIST

NIST Center for Neutron Research

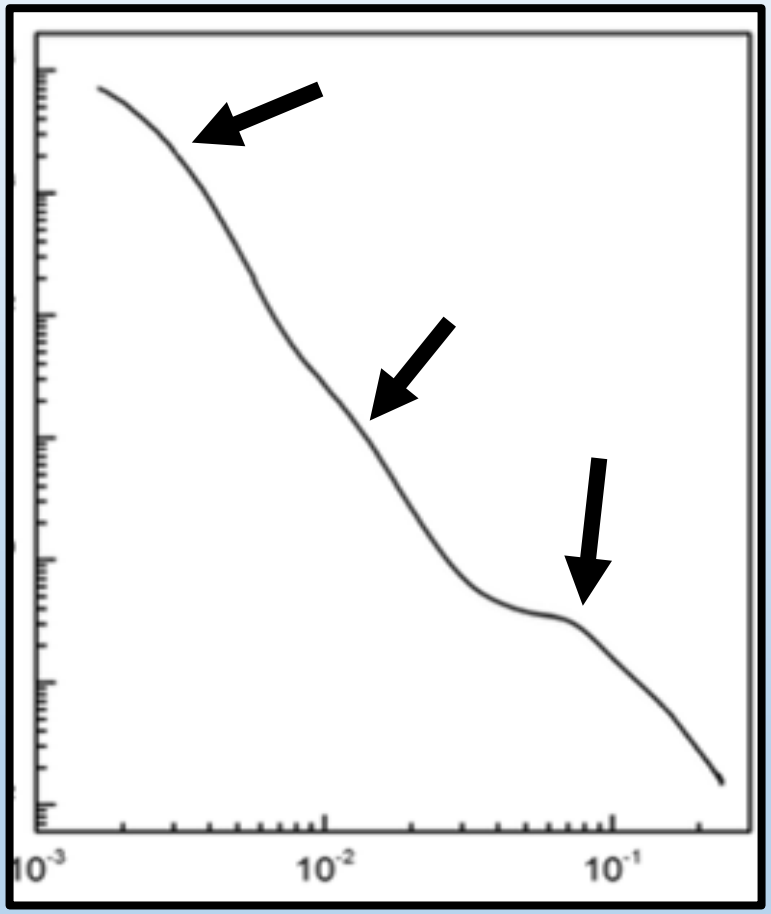

Image from: http://www.borrisoleigh.ie/2009-11-18/

## To contribute towards:

- a better assignment of the casein micelle scattering profile

→ a better understanding of structure and applications ←

Typical Small Angle Scattering of Milk




Adapted from Ingham et. al (2016). *Soft Matter*. 12: 6937-6953

# Milk has been part of the human diet for ~8000 years. What's new?

## We don't fully understand it yet

- None of the current models explains all experimental results for the particles that stabilize milk : **CASEIN MICELLES**

- NIST SRM 1549a was the chosen sample for this project:
  - certified mass fraction values
  - contributions from this project's findings

https://phys.org/news/2009-08-years-central-europe.html

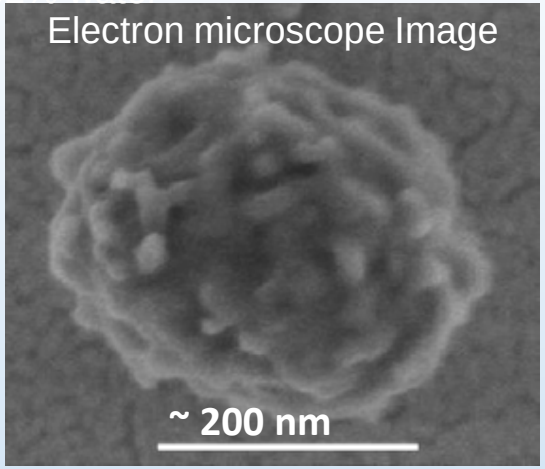
Image from: https://alfafoods.co.nz/wp-content/uploads/2016/01/milk1.jpg

Image from Dalgleish. (2011). Soft Matter (7): 2265-2272.

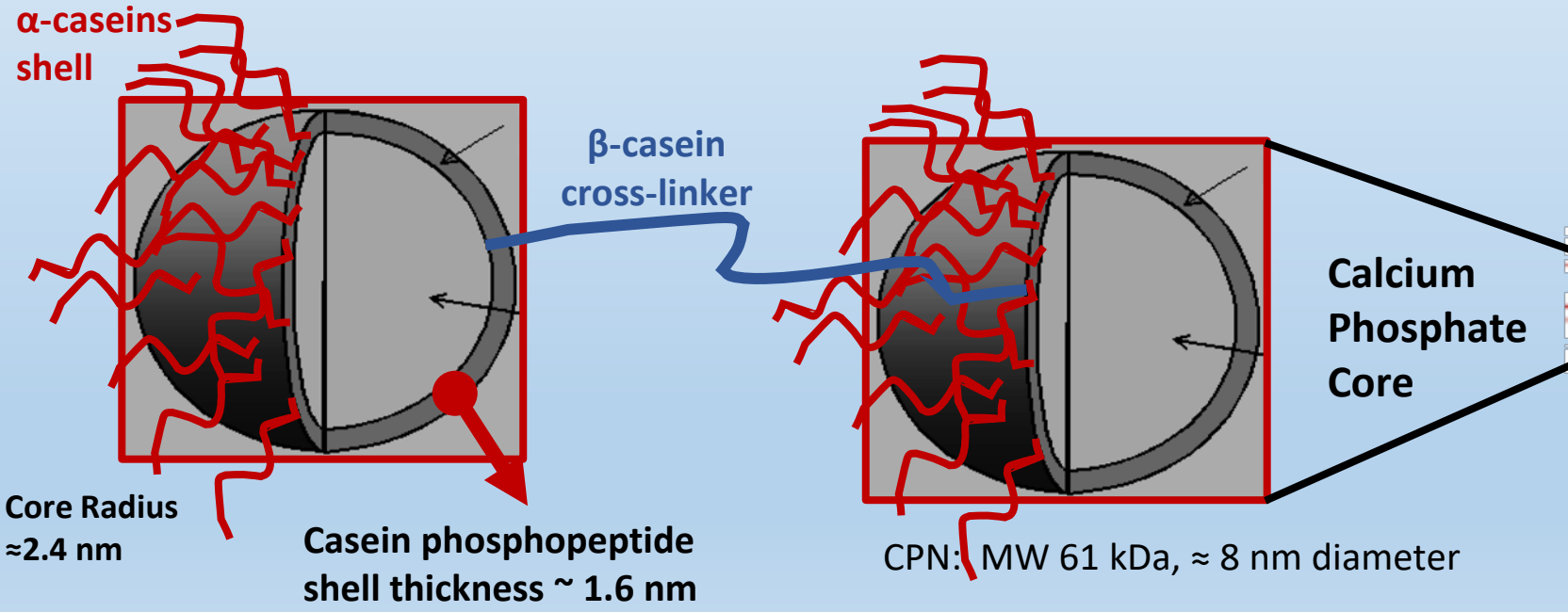
# What are casein micelles?

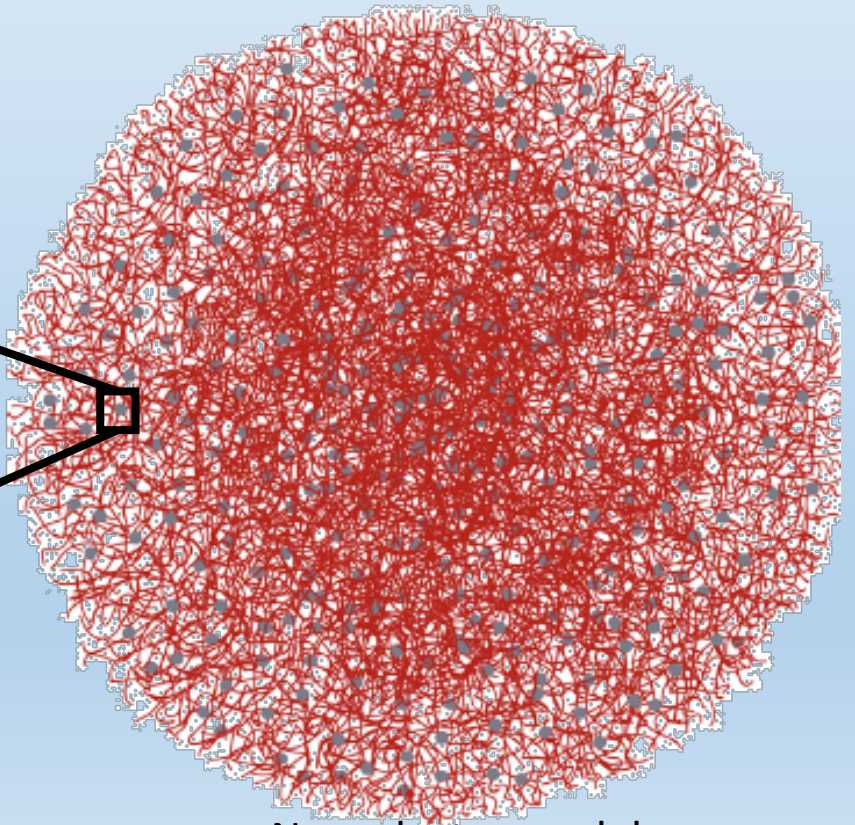
Sub-micron particle with various sizes depending on many factors

MW $2x10^8$ Da
(10 000 peptide chains)
~ 800 CPN




## Calcium Phosphate Nanoclusters (CPN)




Nanocluster model

Adapted from: C. Holt (2016). *International Dairy Journal*. 60: 2-13

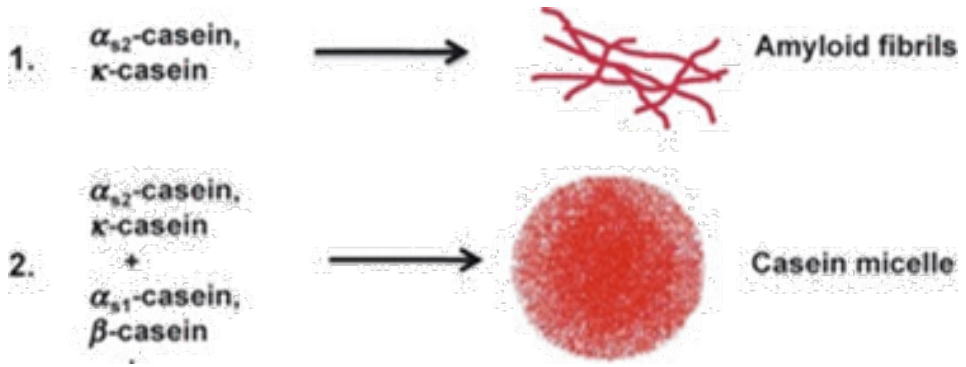
Peter Hristov, et. al. (2016). *Milk Proteins - From Structure to Biological Properties and Health Aspects*

# Function/Applications of Casein Micelles

## Biomineralization

- Milk is supersaturated in calcium phosphate. Caseins stabilize it.

## Inhibit amyloid fibrils




## Drug & Nutrient Carriers

- Biocompatible, biodegradable

## Dairy quality & performance

- Micelle size influences processing and texture of dairy products

Adapted from Holt et. al. (2012). Journal of Evolutionary Biology. 25: 1253–1263

Bouchoux et. al. (2015). Soft Matter. 389-399

# Research Strategy

## Stabilize

**Simulated Milk Ultra Filtrate**

- Buffer mimics milk
- Added natural stabilizers
- Temperature control

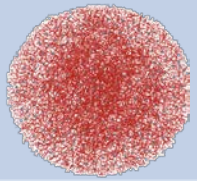
## Simplify

Skim the milk

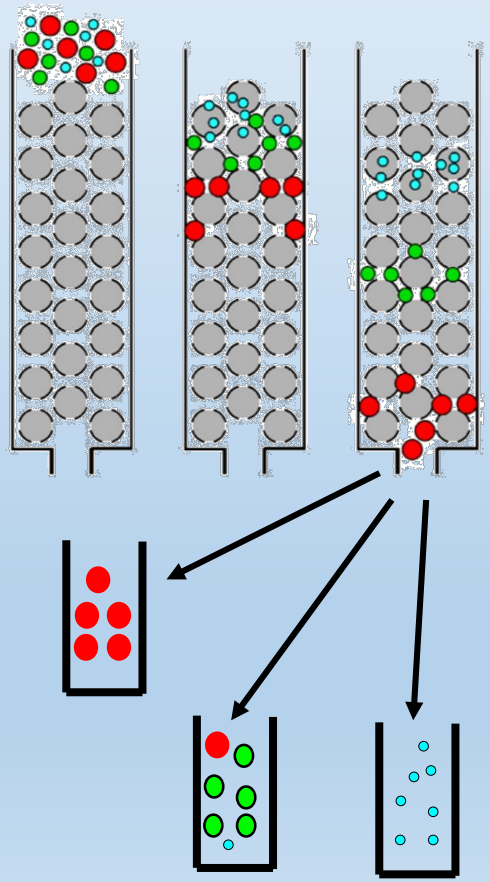
Fractionate the Casein Micelles

Use SANS w/ contrast variation:

CPN



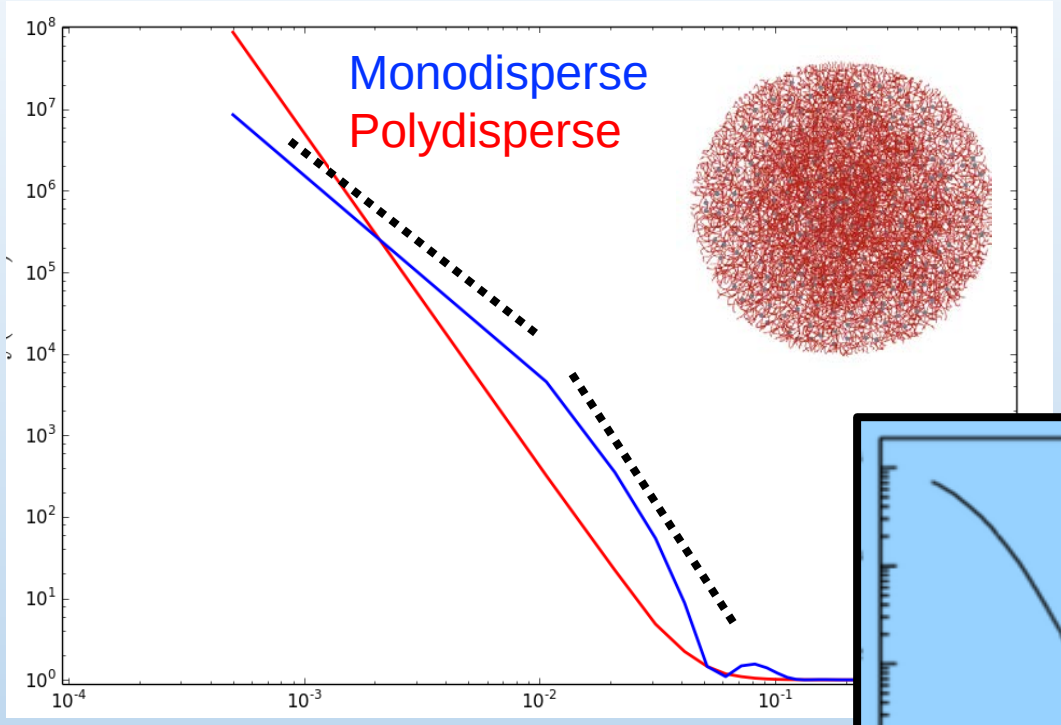
CM

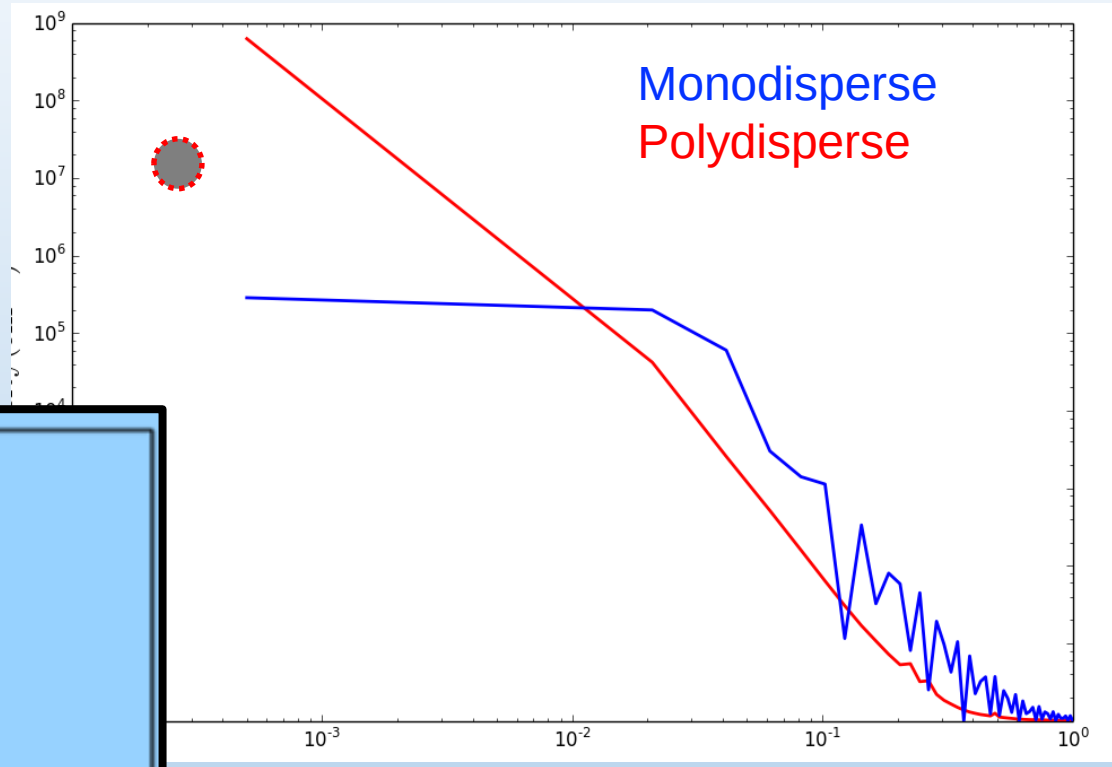
Adapted from: http://www.creative-biostructure.com/custom-size-exclusion-chromatography-service-259.htm

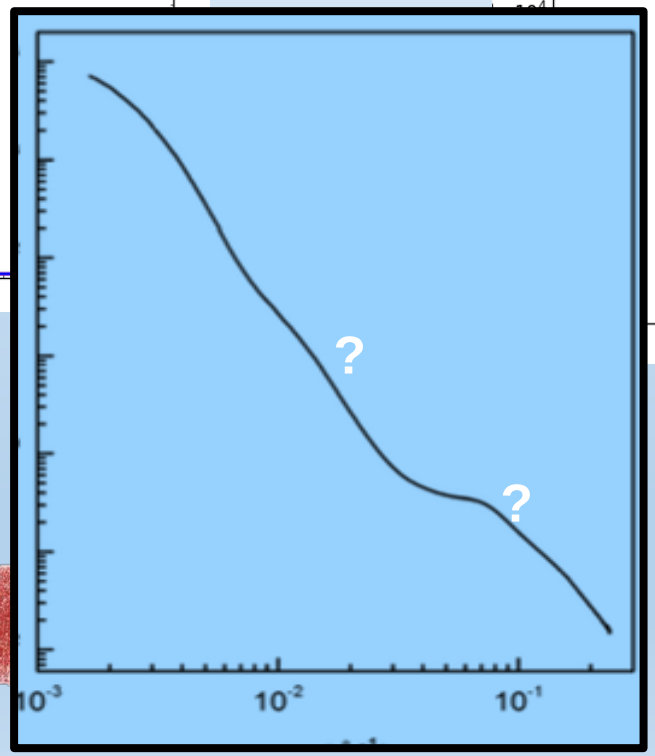
# Why neutrons?

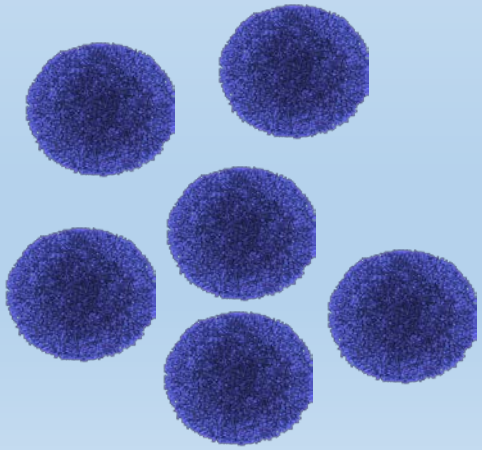
**Simulation**
**Fuzzy Sphere R=150 nm (CM)**



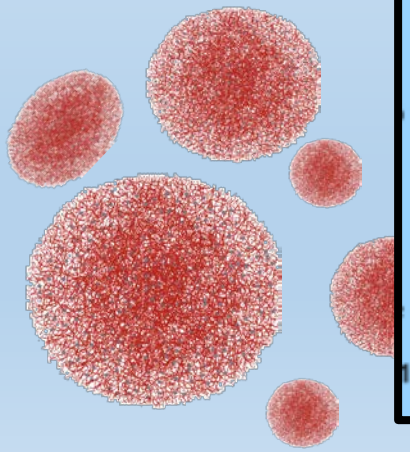

**Simulation**
**CPN Nanoclusters R= 4 nm**



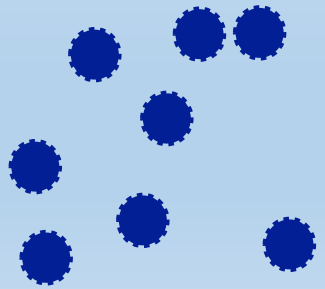



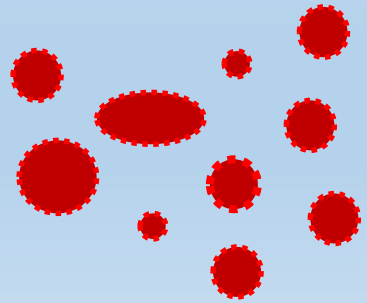
Monodisperse



Polydisperse

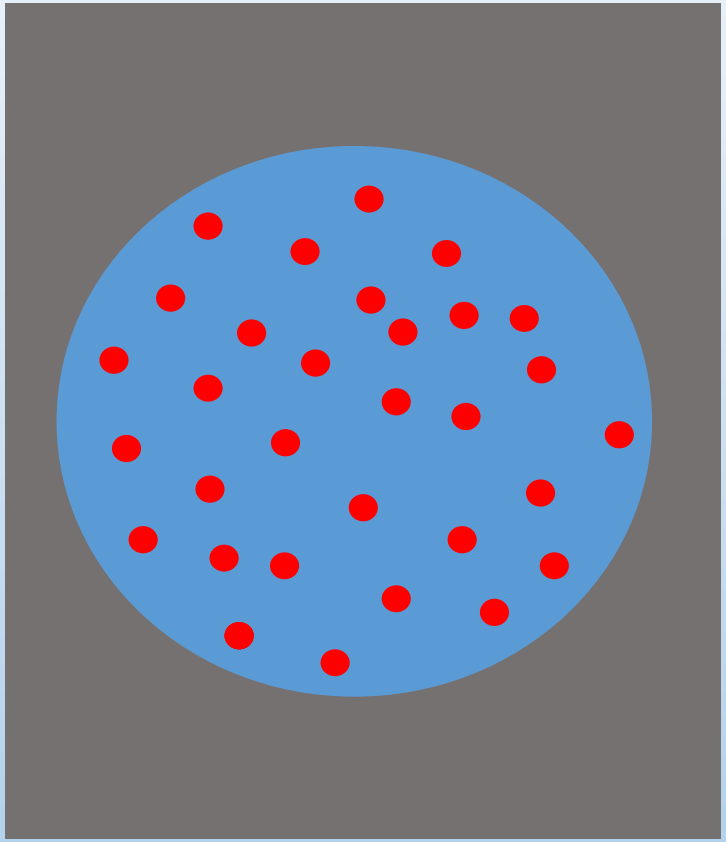


Monodisperse

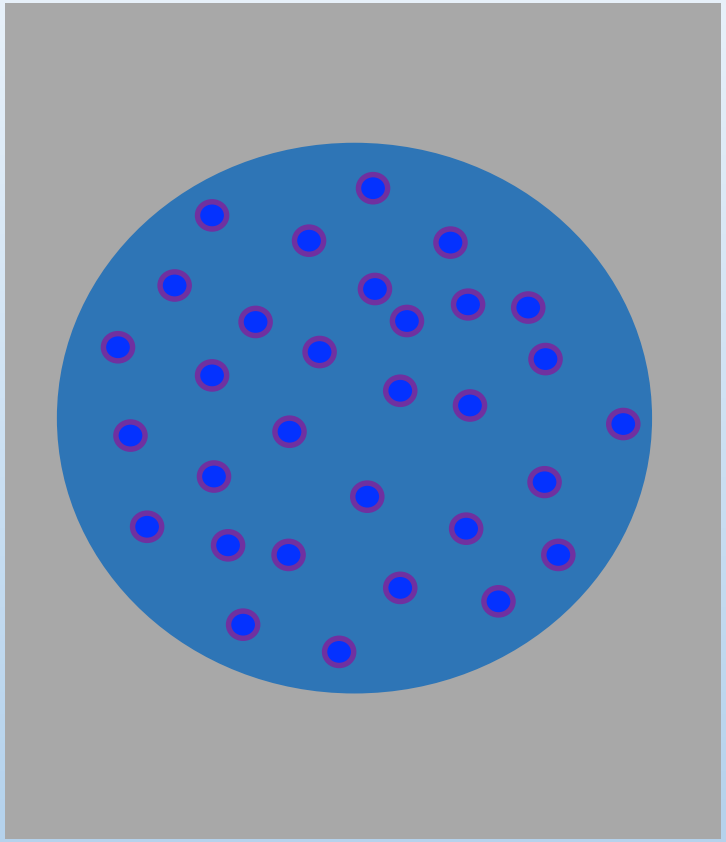


Polydisperse

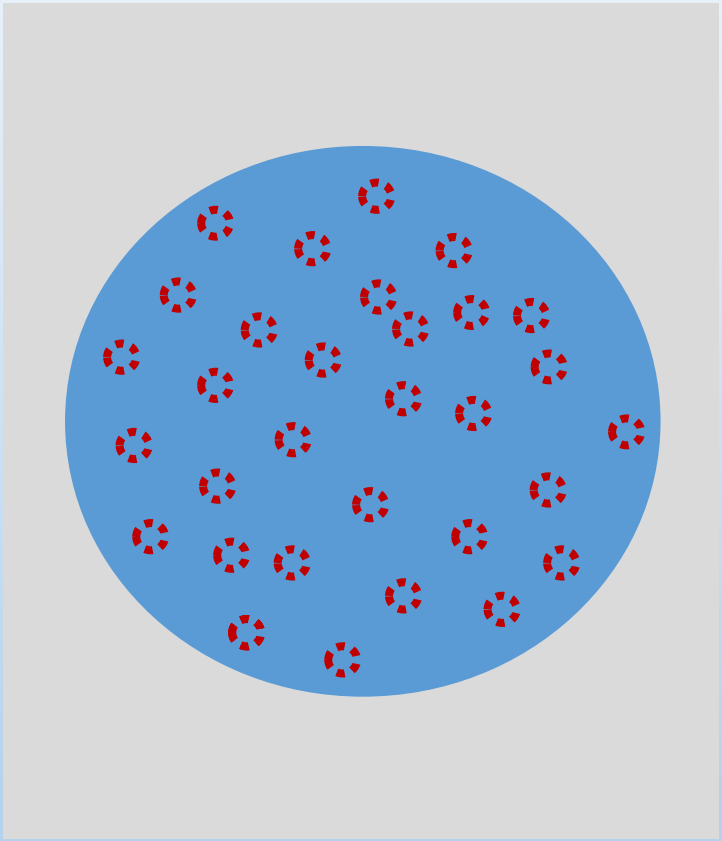
# Why neutrons?



$H_2O$



70% $D_2O$

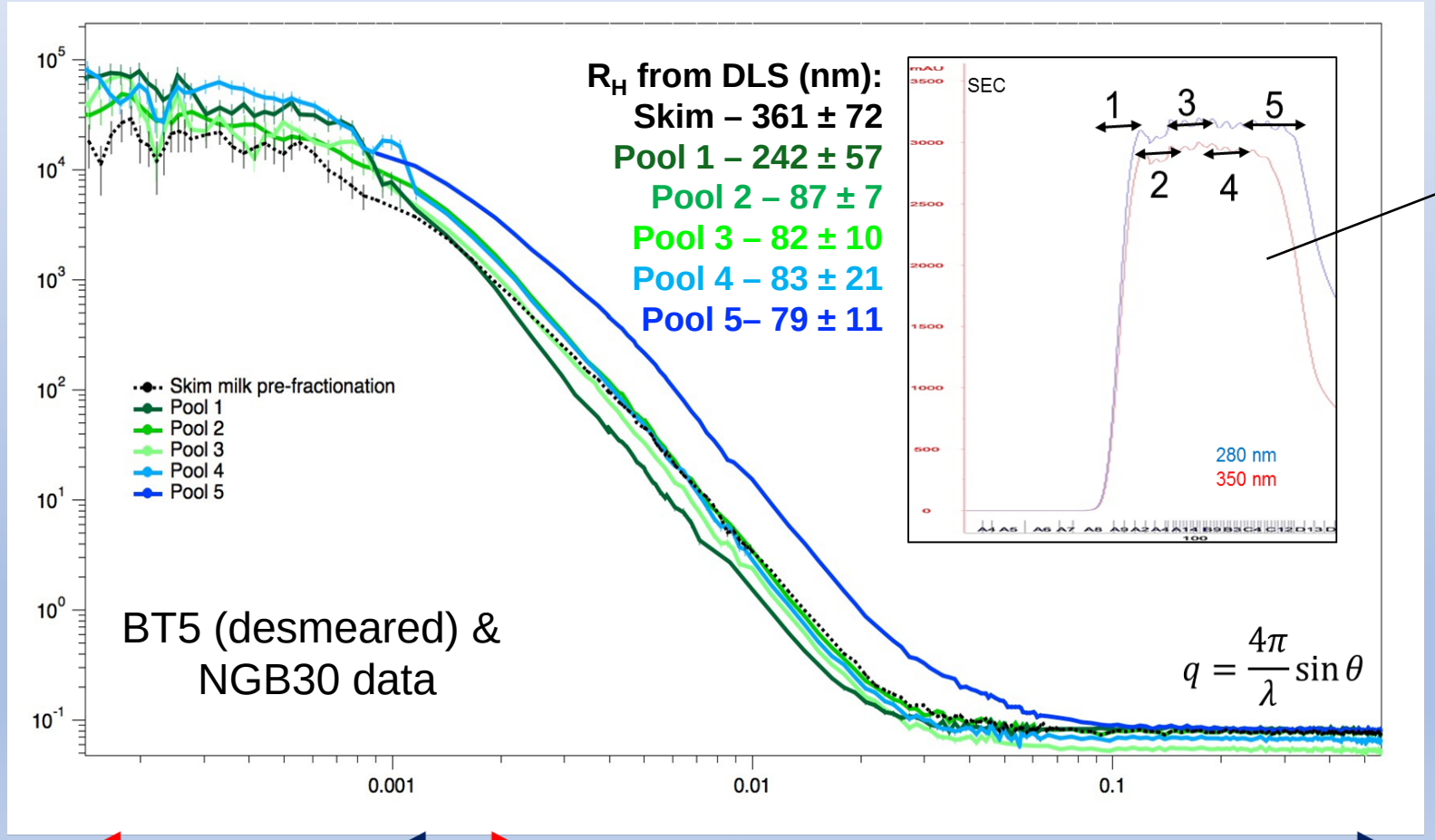


98% $D_2O$

# Small Angle Neutron Scattering (SANS)

## SANS is the least intrusive, most informative technique

- Covers broad range of angles
- Does not require cooling, additives, etc. (less artifacts from sample preparation)




UV-Visible Signal easily saturated

Used instead:

- UV-vis of diluted fractions
- Dynamic Light Scattering

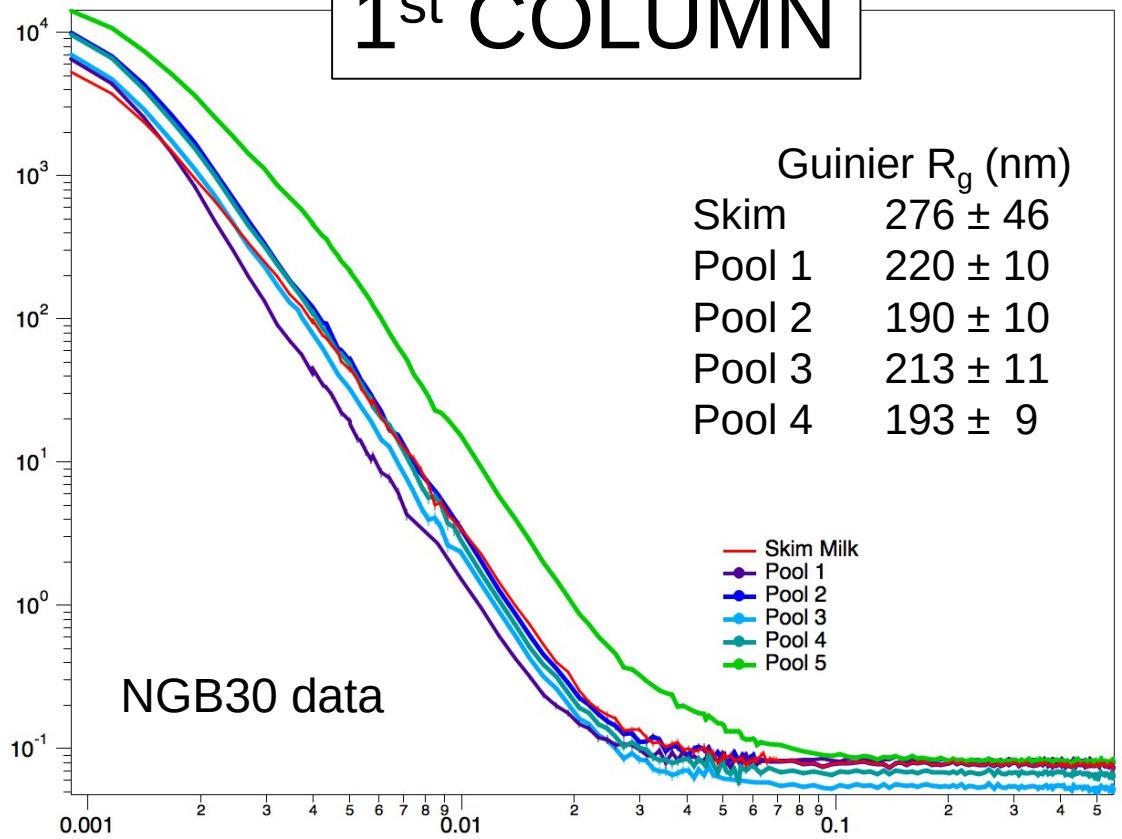
"Reduction and Analysis of SANS and USANS Data using Igor Pro", Kline, S. R. J Appl. Cryst. 39(6), 895 (2006).

# Fractionation helps – Optimization of SEC runs

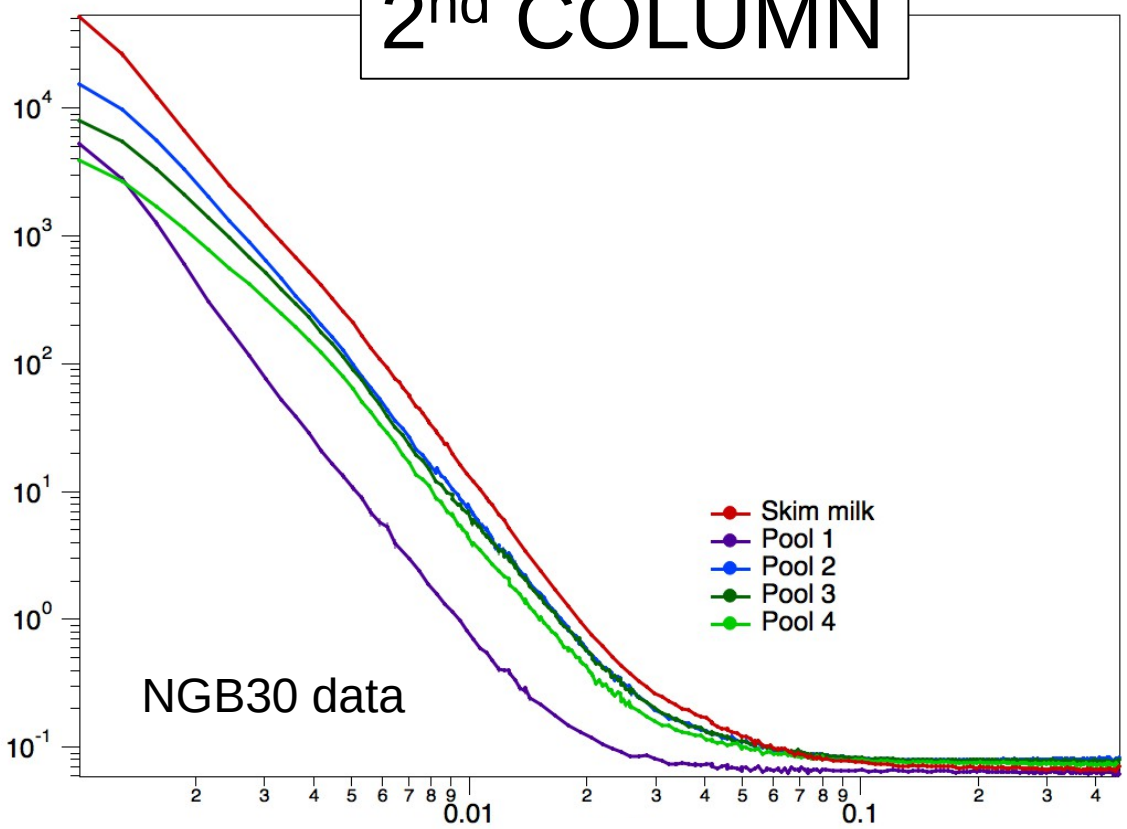
Faster flow, 2ml fractions, more fractions pooled together. Run at 4℃

Less column saturation, 0.5 ml fractions, less volume pooled together. Run at 10℃

## 1st COLUMN

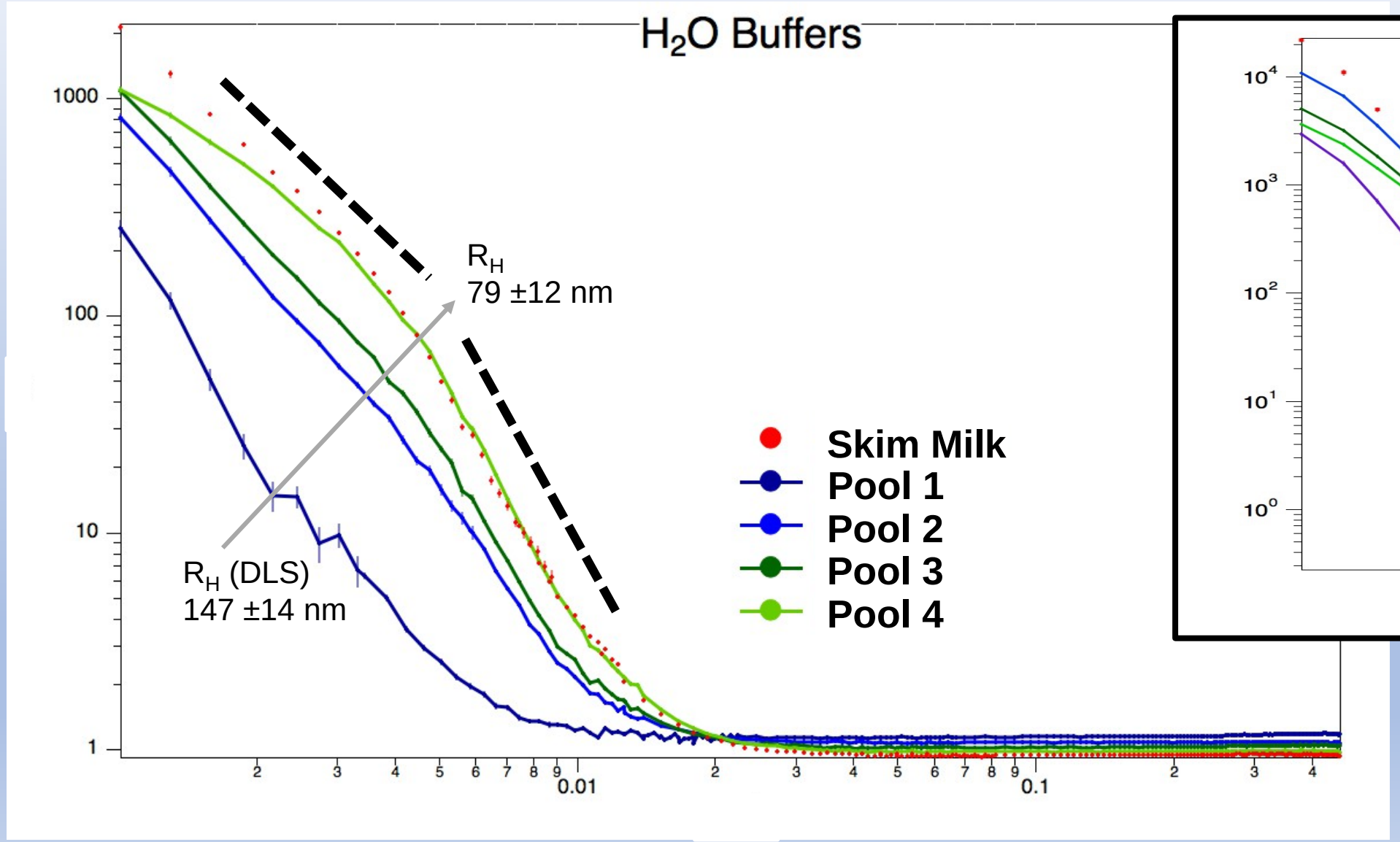


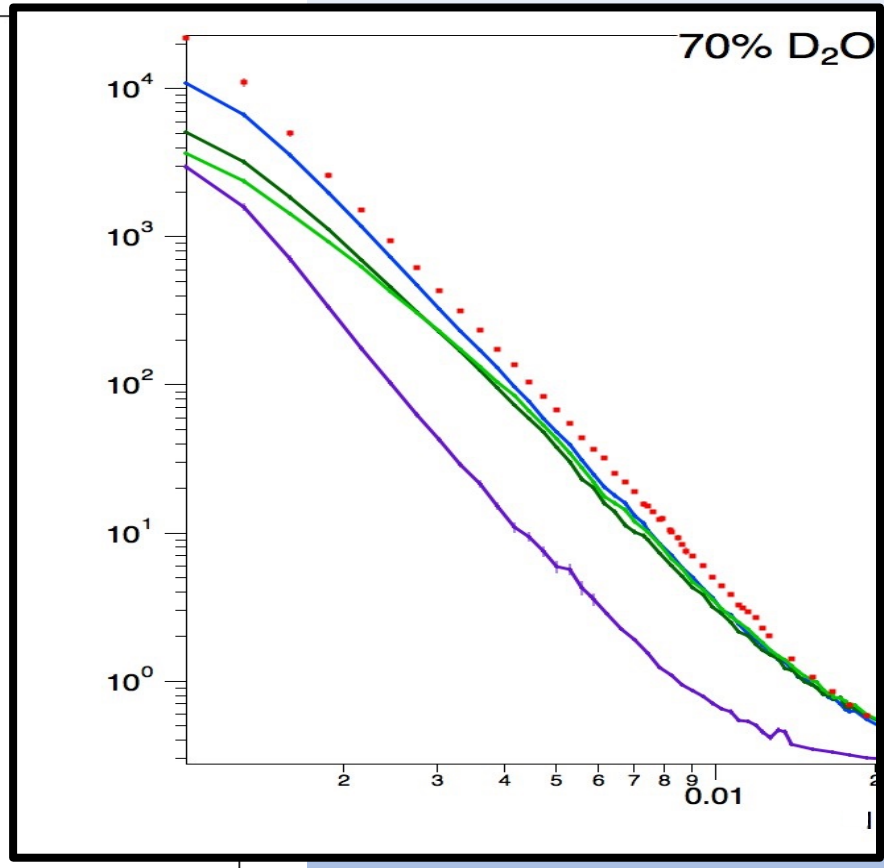

## 2nd COLUMN




SANS profiles in $D_2O$ buffers have better signal-to-noise but also match out the CPN contributions. The change in size distributions is however still clear.

# Fractionation helps – Contrast Variation

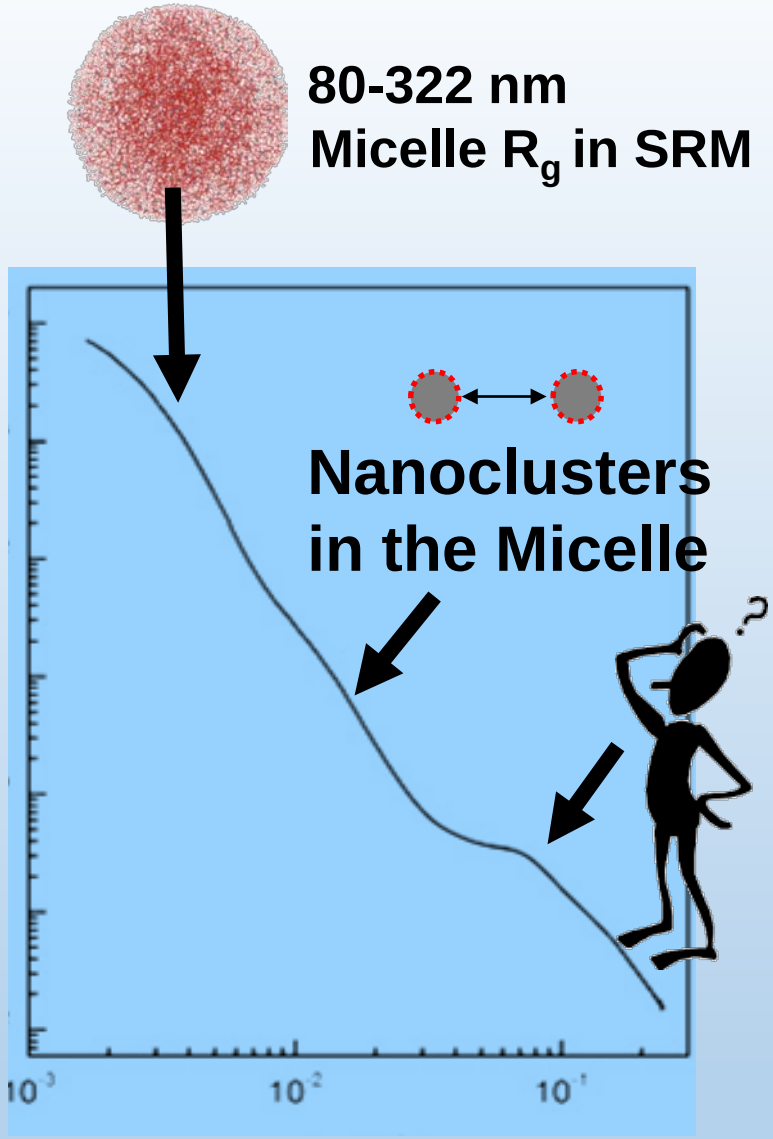


# Conclusions

- SEC can reduce the natural polydispersity of milk for improved resolution
- Mid-q feature of the spectrum is matched out in $D_2O$ buffers (CPN matched out).
- USANS-SANS provides information on the different components but sample monodispersity can only be kept for 1-2 days.

***VSANS please!***



# Acknowledgments


- S. Teixeira (SURF Mentor, U. Delaware/NIST)
- T. Hoopes (IBBR), T. Cleveland, & M. Shremshock (NIST)
- L. Wood (MML Material Measurements Laboratory)
- B. Brooks (RET program)
- NCNR SURF team: J. Dura & J. Borchers

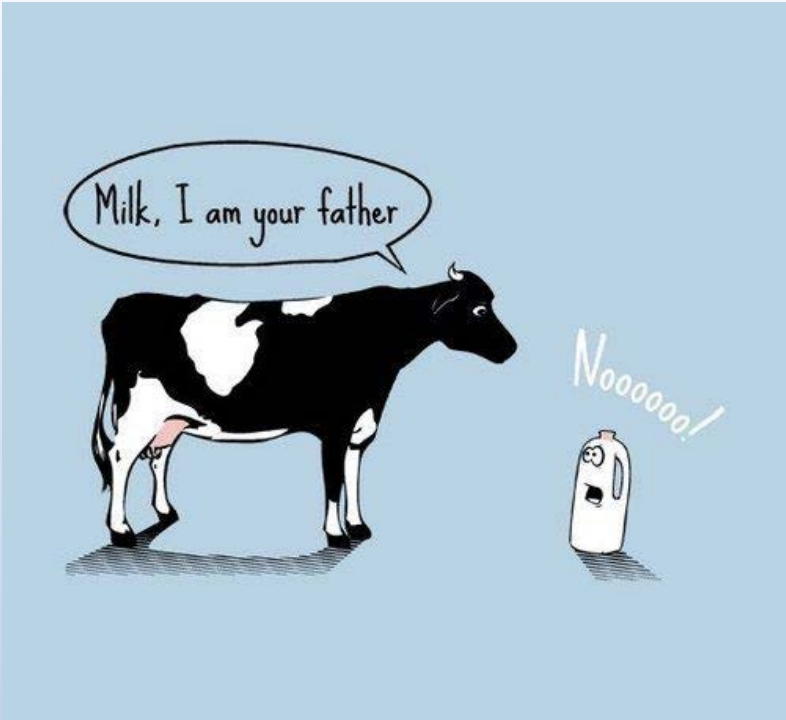




Image from: https://www.snorgtees.com/milk-i-am-your-father

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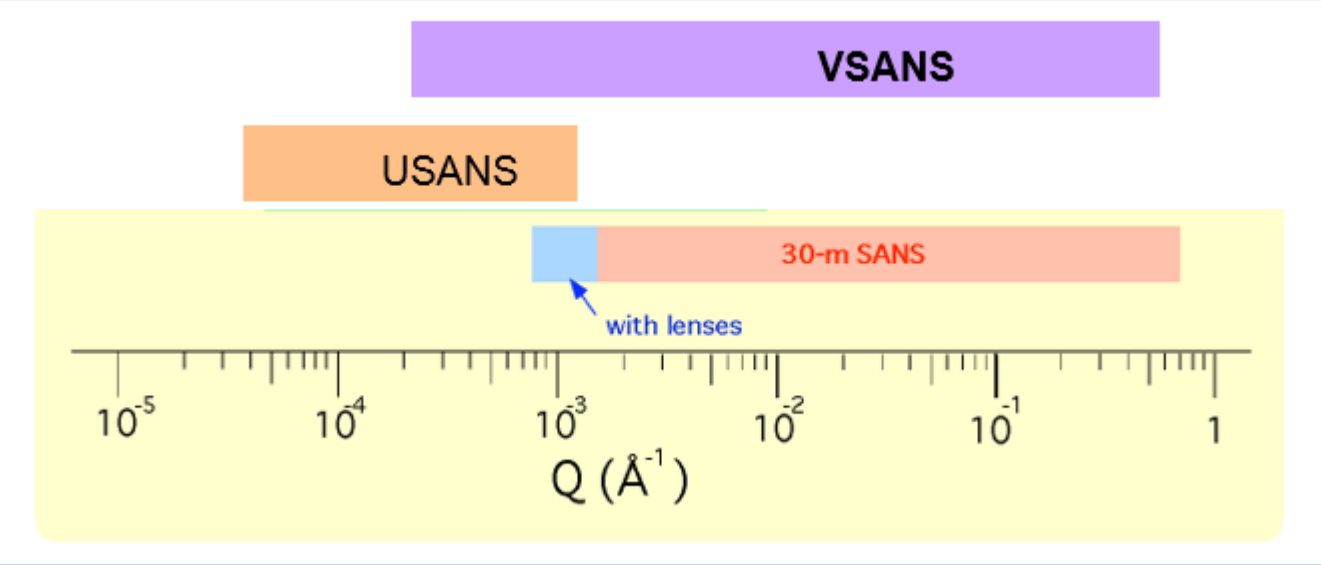
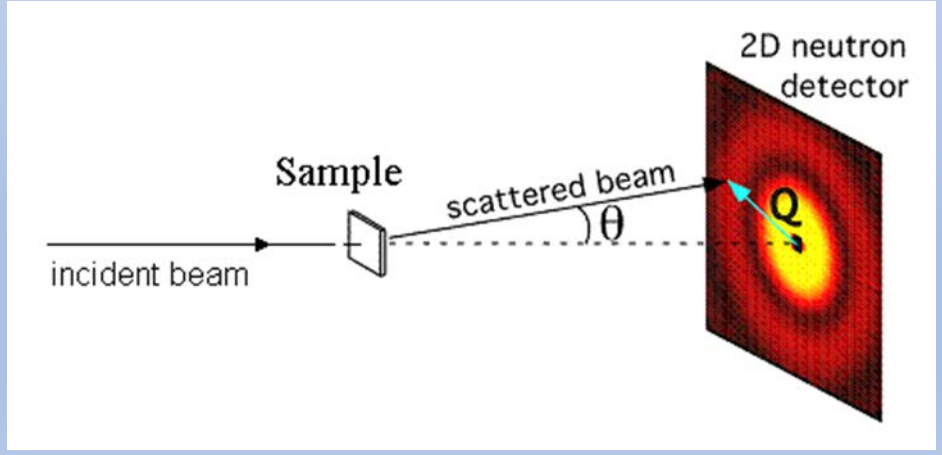
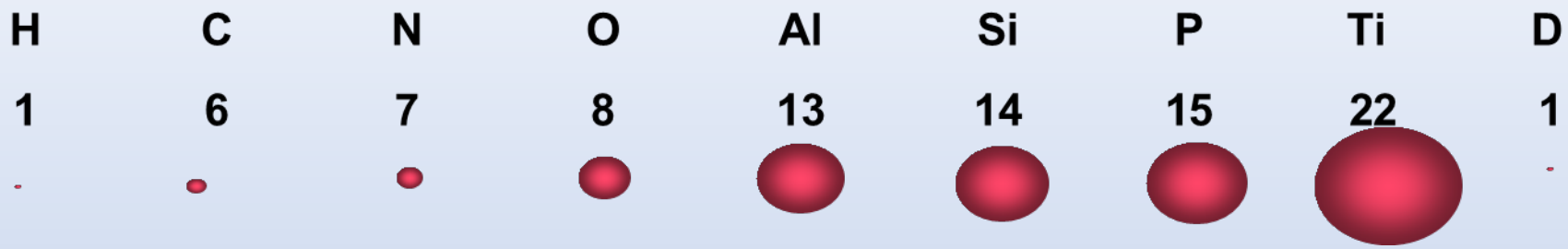
NIST Center for Neutron Research


VSANS
USANS
30-m SANS
with lenses
10-5
10-4
10-3
10-2
10-1
1
Q (Å-1)


2D neutron
detector
Sample
scattered beam
θ
Q
incident beam



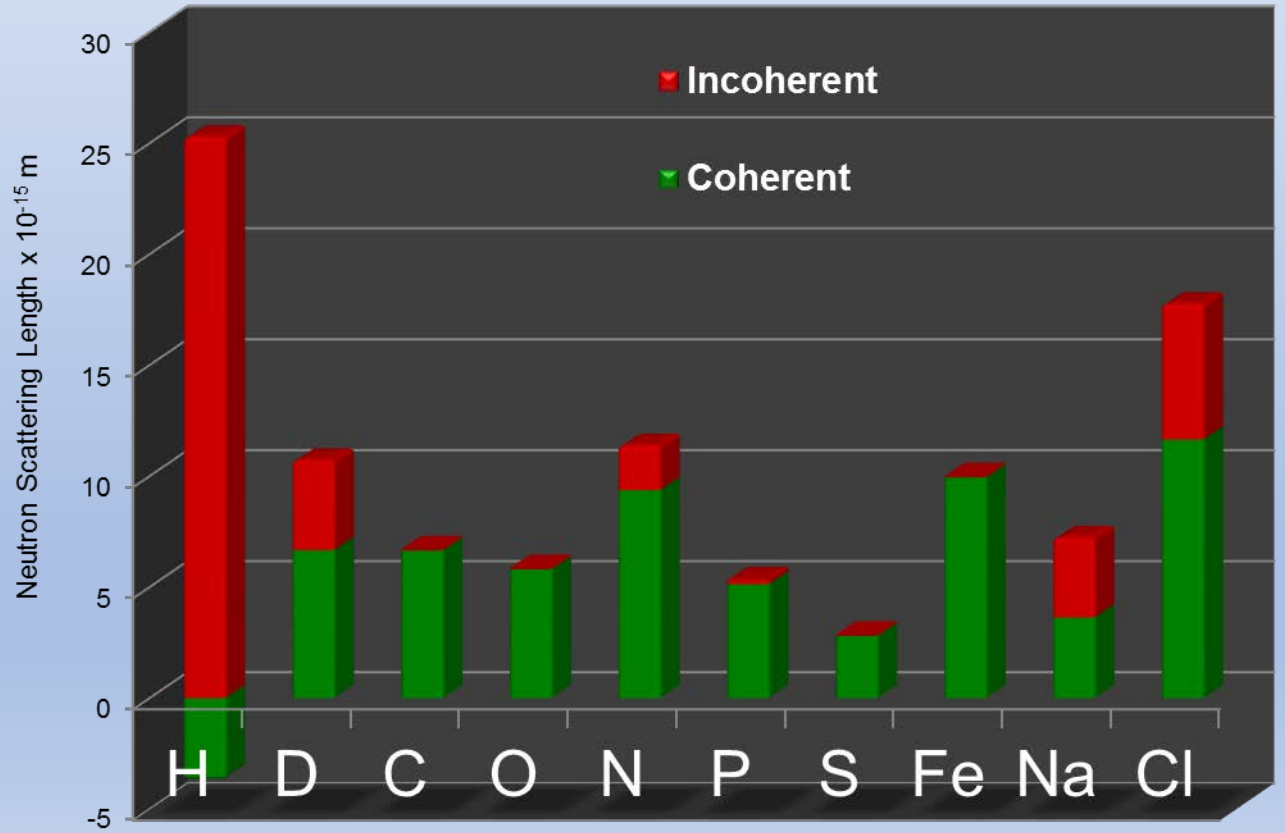
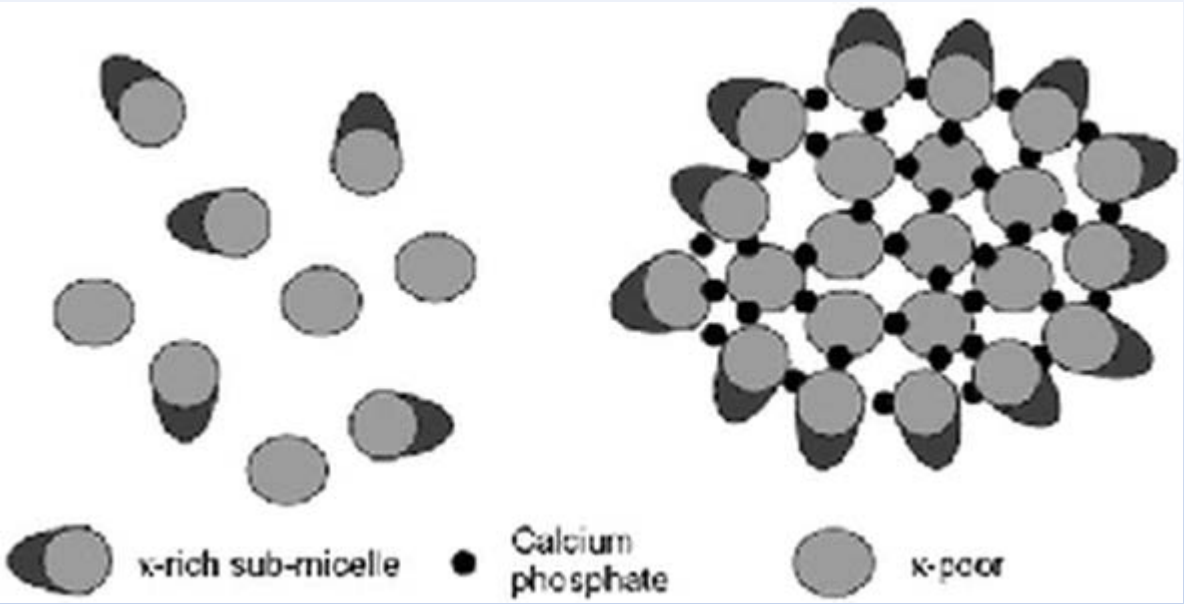
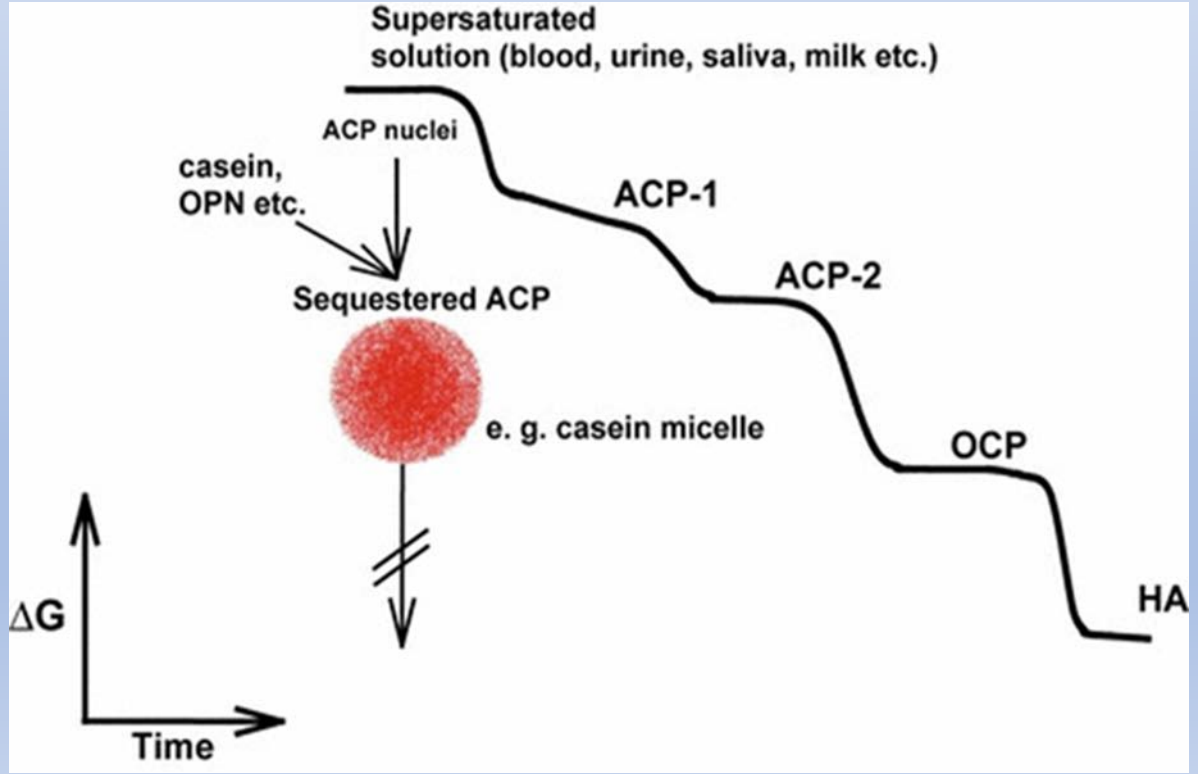



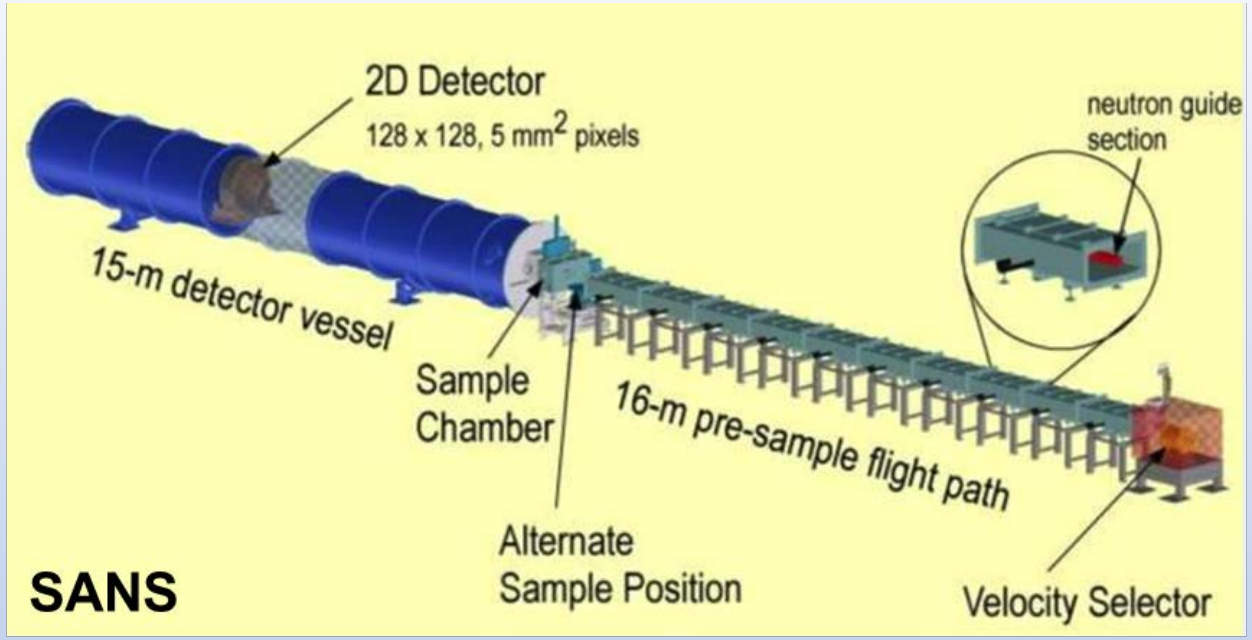

Image credit: Susana Teixeira (NIST)

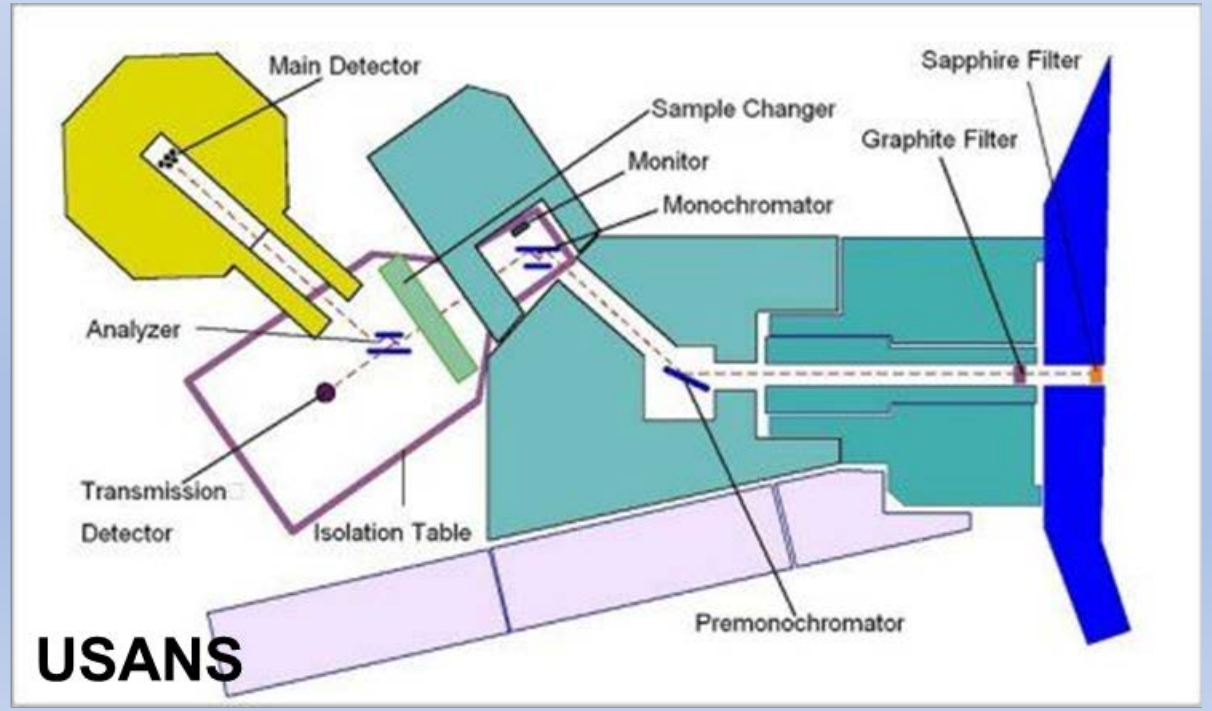


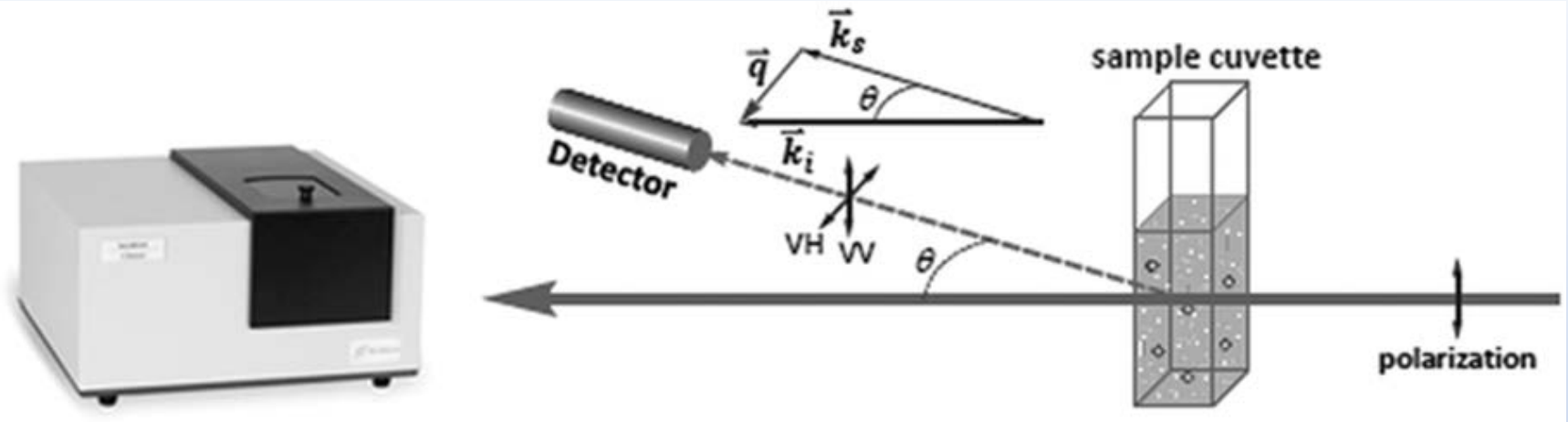

Peter Hristov et. al (2016). Measurement of Casein Micelle Size in Raw Dairy Cattle Milk by Dynamic Light Scattering, Milk Proteins - From Structure to Biological Properties and Health Aspects

Holt et. al (2012). Darwinian transformation of a 'scarcely nutritious fluid' into milk. *Journal of Evolutionary Biology*

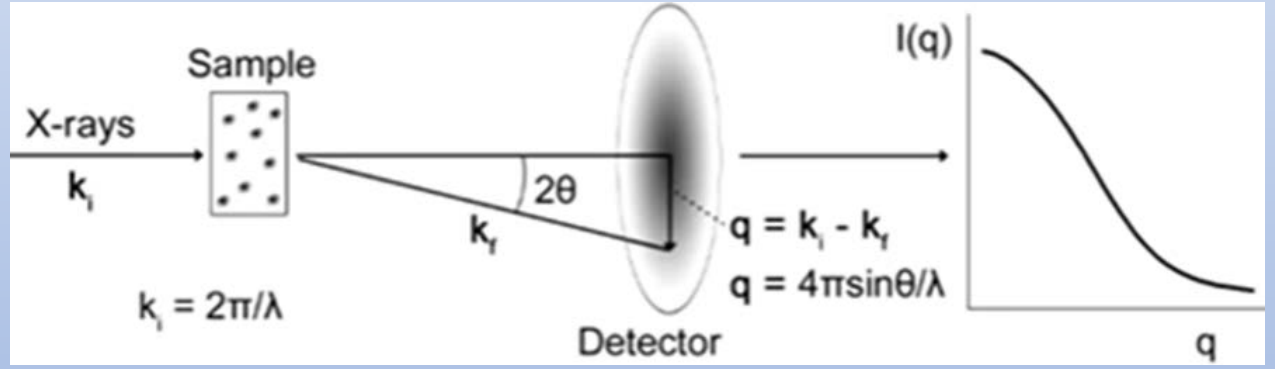



2D Detector
128 x 128, 5 mm2 pixels
neutron guide section
15-m detector vessel
Sample Chamber
16-m pre-sample flight path
Alternate Sample Position
Velocity Selector
SANS


Main Detector
Sapphire Filter
Sample Changer
Graphite Filter
Monitor
Monochromator
Analyzer
Transmission Detector
Isolation Table
Premonochromator
USANS




Peter Hristov, et. al. (2016). Measurement of Casein Micelle Size in Raw Dairy Cattle Milk by Dynamic Light Scattering, Milk Proteins - From Structure to Biological Properties and Health Aspects




- Size Exclusion Chromatography: Separation by Size
  - Akta Purifier System
  - Hiprep 26/60 Sephacryl S-500 Column (Matrix: cross-linked copolymer of allyl dextran and N,N'-methylene bisacrylamide)