# Engineered Nanoparticles in Agricultural Settings

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# Antimicrobial NPs: Many Applications

- Paints
- Textiles
- Medicine
- Washing machines
- Food packaging, water containers
- Cosmetics
- And a growing range of unsubstantiated uses…

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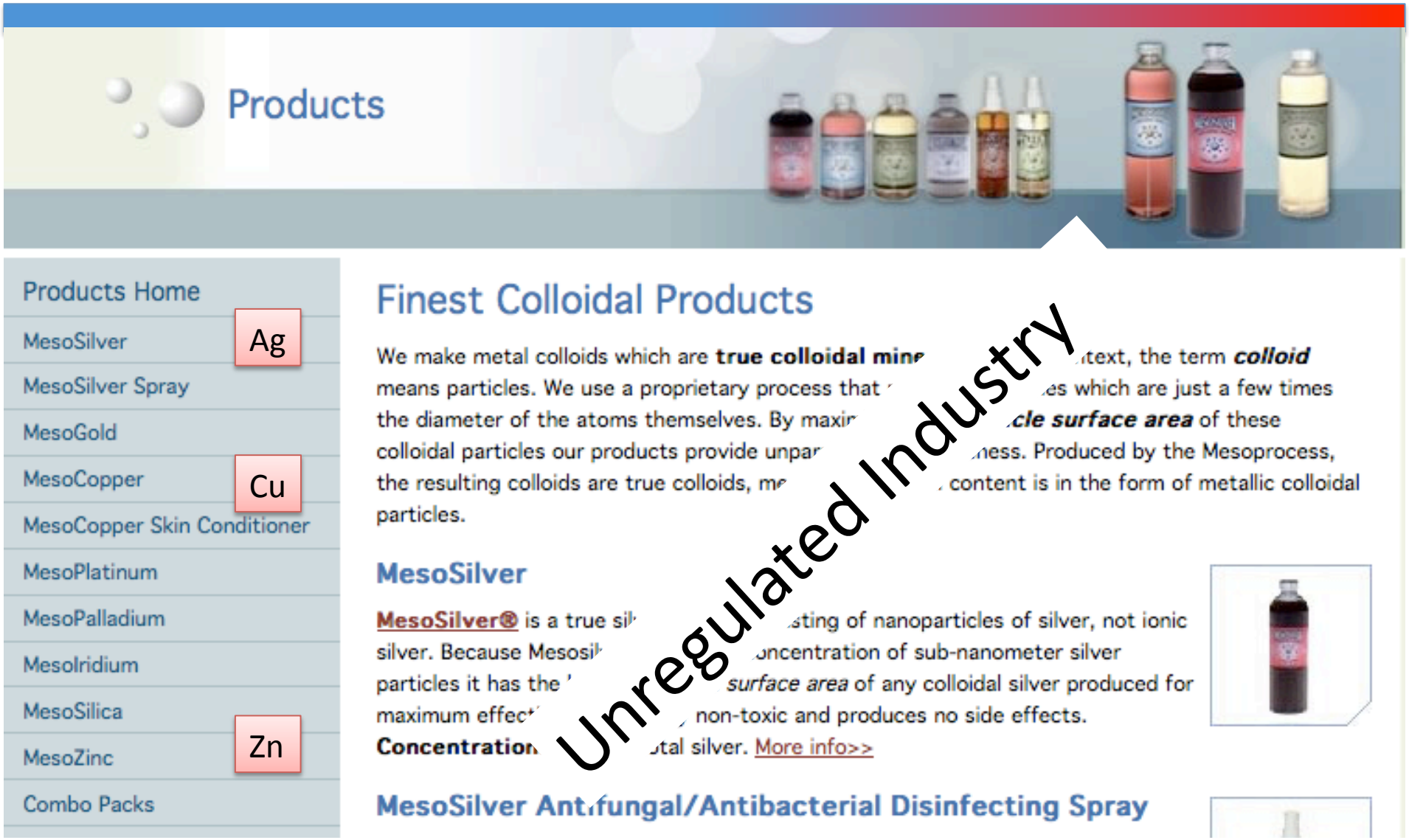
# NPs (&/or ions): At a store near you



# Antimicrobial NPs Studied Here

Ag

Cu

Zn

- Silver: Ag (~10nm)*
- Copper Oxide: CuO (~33nm)*
- Zinc Oxide: ZnO (~70nm)*
  - All commercial formulations
  - No surfactants or stabilizing agents
  - Ag NPs laser synthesized from metal in water
- Our focus:
  - <u>Modes</u> of interaction with wheat and root colonizing soil microbes (*Pc*O6) and <u>characterizing</u> NPs

* Nominal sizes from manufacturers (NLC labs, Sigma Aldrich)

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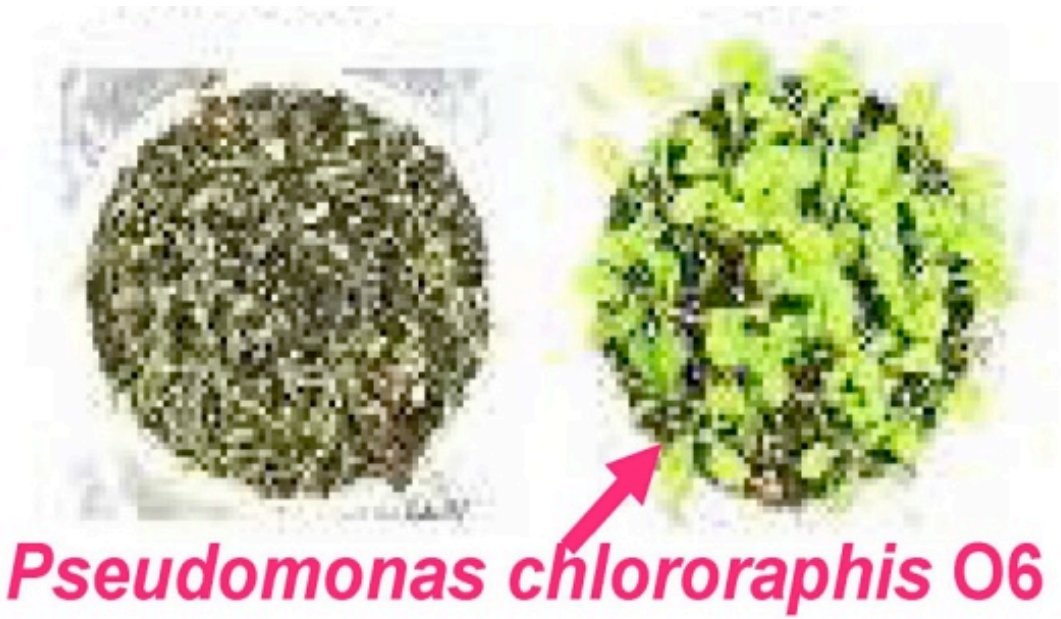
# *Pc*O6: Beneficial Root Microbe

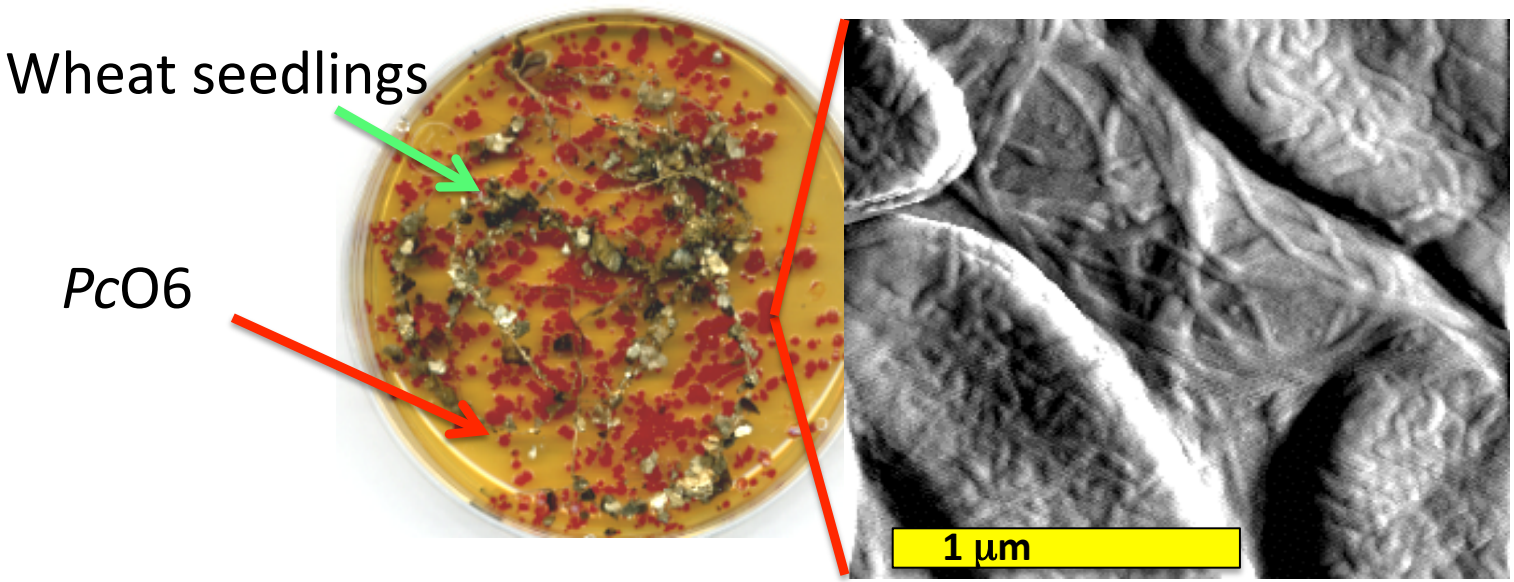
Drought Tolerance Imparted by Microbe to Host Plant:




Cho S.M., Kang B.R., Han S.H., Anderson A.J., Park J.Y., Lee Y.H., Cho B.H., Yang, K.Y., Ryu C.M., & Kim Y.C. (2006). *Molecular Plant Microbe Interaction, 21, 1067-1075.*

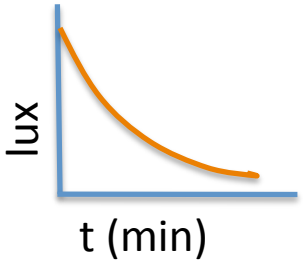
Spencer, M., Ryu, C.M., Yang, K.Y., Kim, Y.C., Kloepper, J.W., & Anderson, A.J. (2003).. *Physiological and Molecular Plant Pathology. 63, 27-34.*

# *Pc*O6 Isolation



# Methods

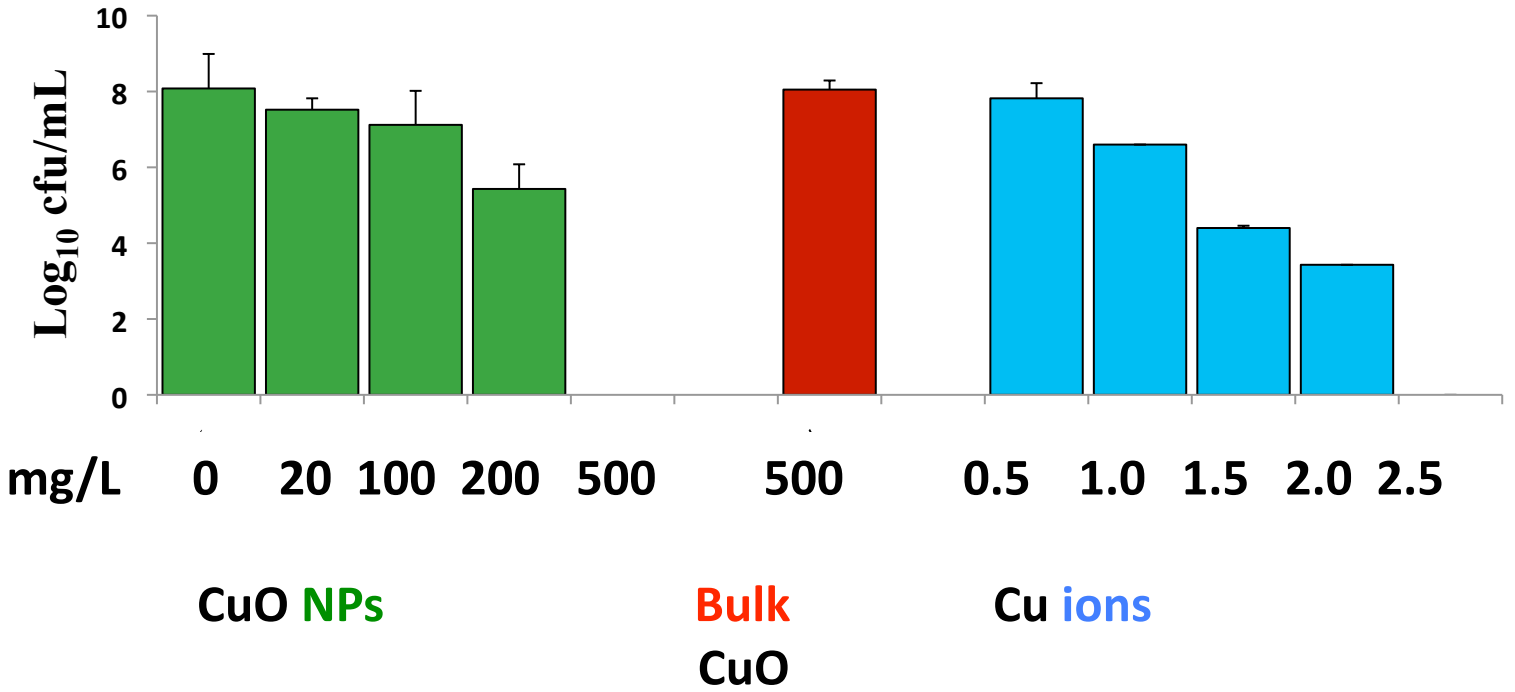
- NP Characterization
  - Field flow fractionation (FFF), ICP-MS
  - Atomic force microscopy (AFM)
- Biological sensors:
  - Soil microbes with *lux* gene insertions*
  - Standard plate counts




- Biochemical assays for modes of NP activity
  - Reactive oxygen species (ROS) enzymes and metabolites -- MDA and GSSG / peroxidases and catalase
  - Secondary metabolite up / down regulation
    - additional interest: metabolite biofertilizers:
      - indole acetic acid (IAA)
      - Siderophore, pyoverdine (PVD)

* Gajjar et al., J Biol Eng. 2009 Jun 26;3:9.

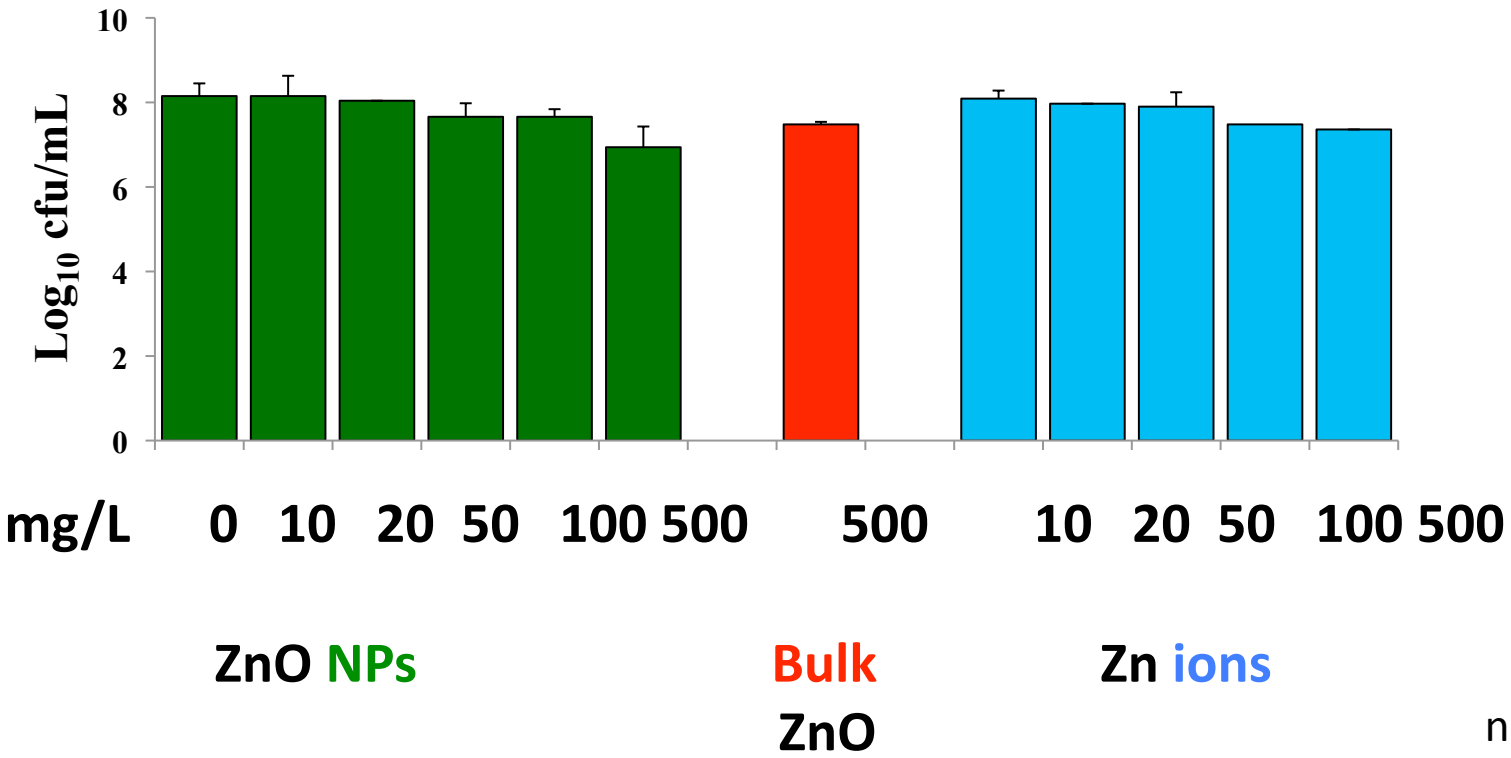
# CuO Toxicity to *Pc*O6




n=3

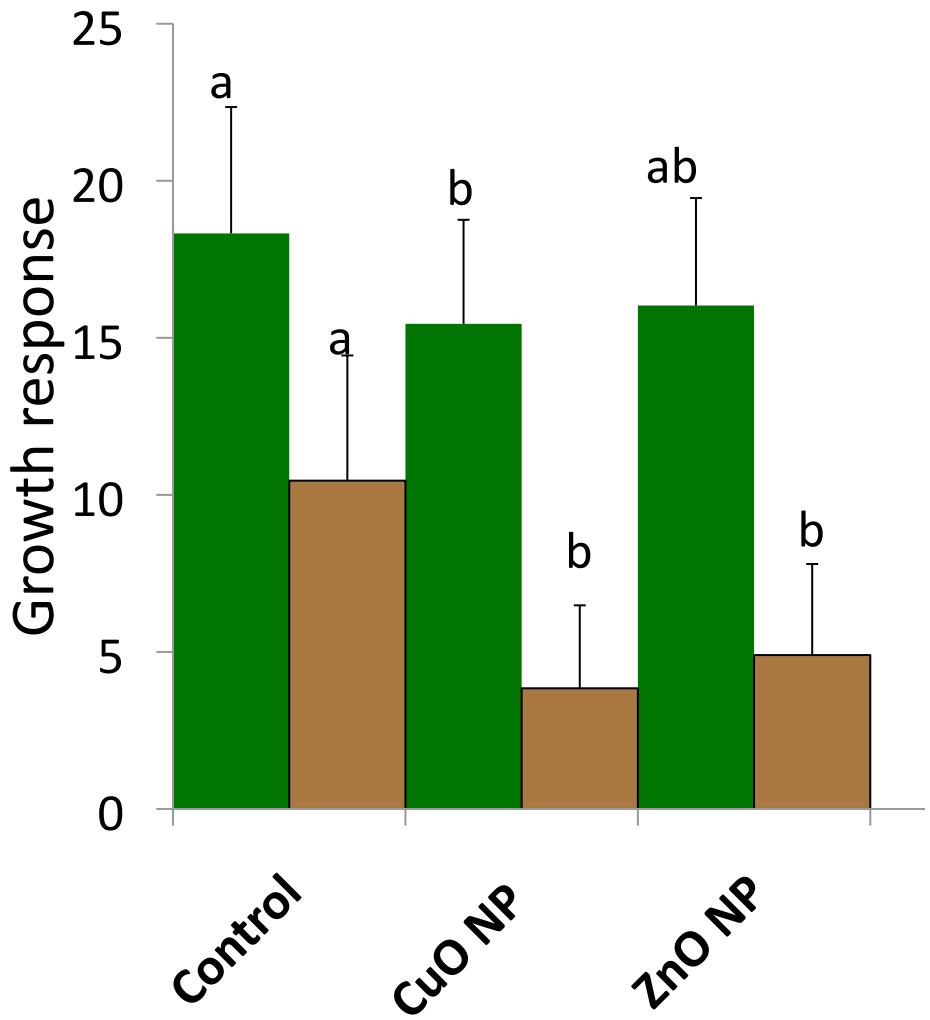
Dimkpa et al., submitted, 2010

# ZnO Toxicity to *Pc*O6




Dimkpa et al., submitted, 2010

# NP Influence on Wheat




**Shoot Length (cm)**

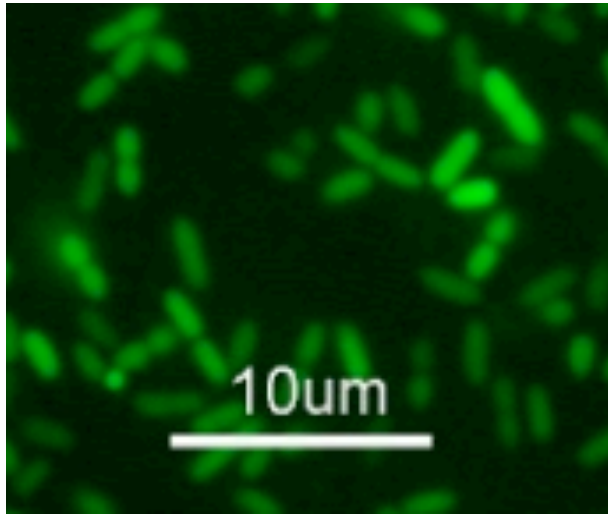
**Root Length (cm)**

- Sand medium
- 500 mg NPs /kg sand
- 14 days
- n = 75

Dimkpa et al., submitted, 2010

# Modes of NP Biocidal Activity

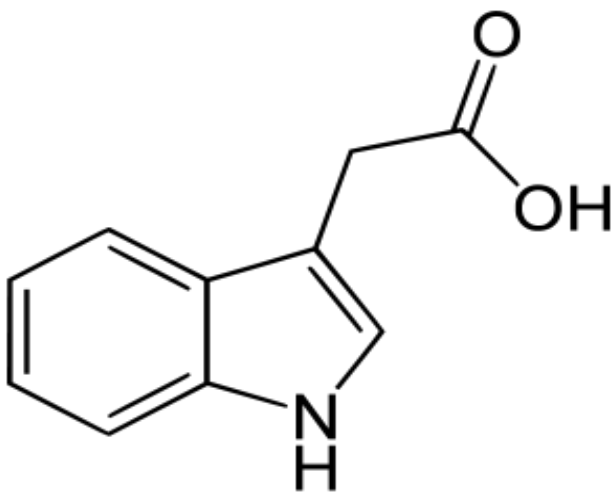
- Not through cell membrane disruption as determined by GFP *Pc*O6 remaining intact following challenges with lethal NP doses




- Biochemical assays…

Dimkpa et al., submitted, 2010

# IAA, a Phytohormone

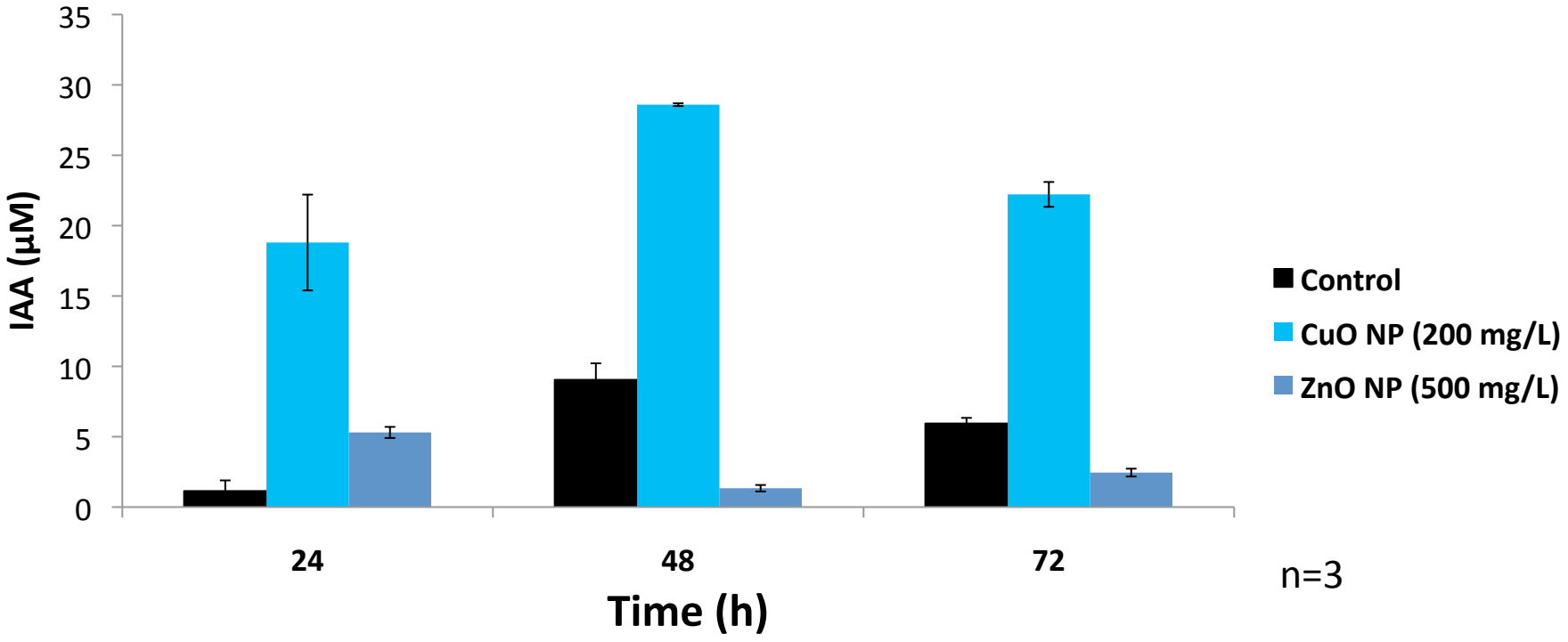



IAA promotes root and shoot growth

Control + Bacterial IAA

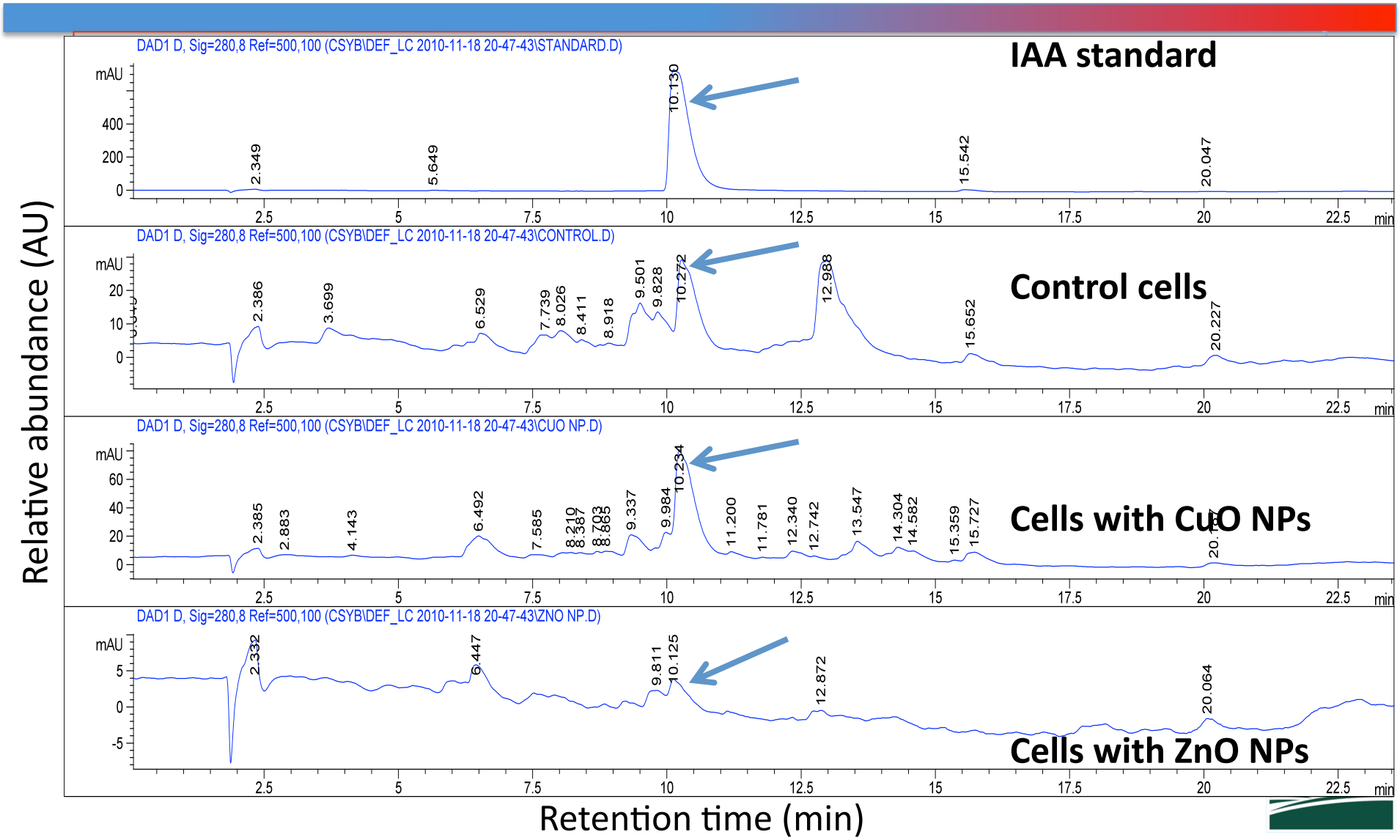
Dimkpa et al., 2008 (Soil Biol. Biochem)

# IAA biosynthesis in *Pc*O6 in the presence of NPs

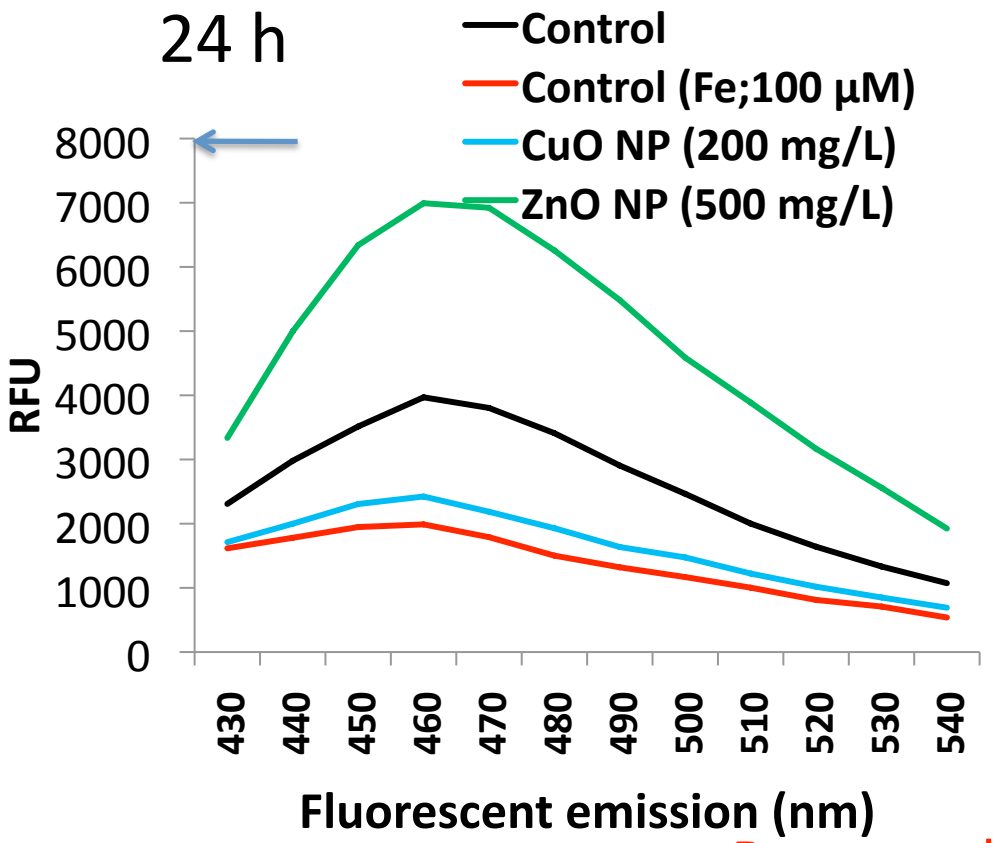


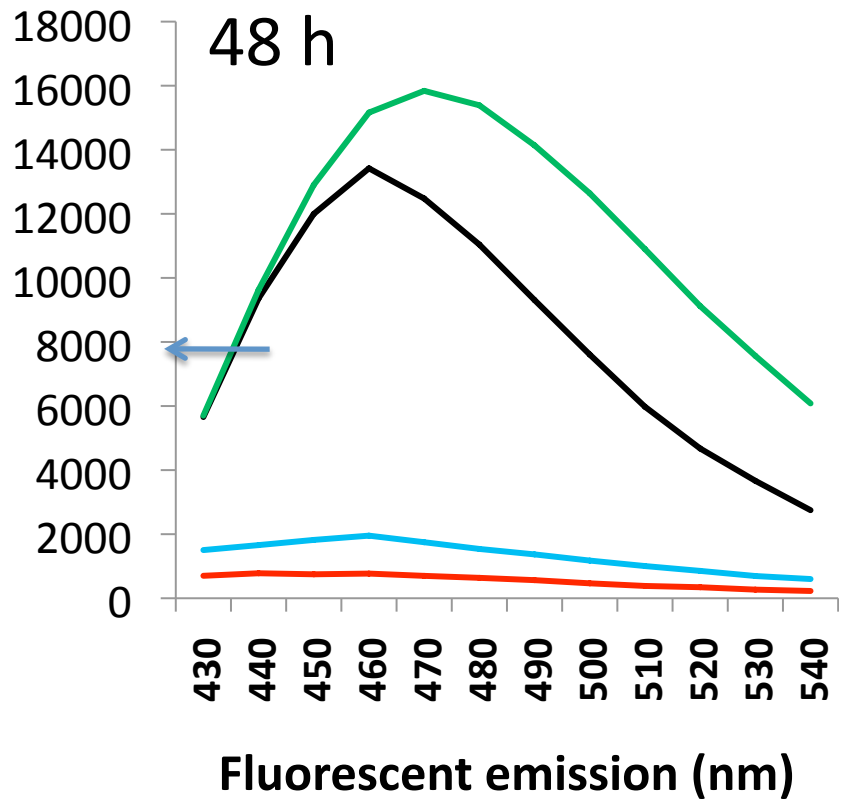

**CuO NPs stimulate IAA production**
**ZnO NPs enhance early IAA production then inhibits**

# HPLC Confirmation of NP up / down Regulation of IAA



# Kinetics of siderophore (PVD) biosynthesis in *Pc*O6: Influence of NPs

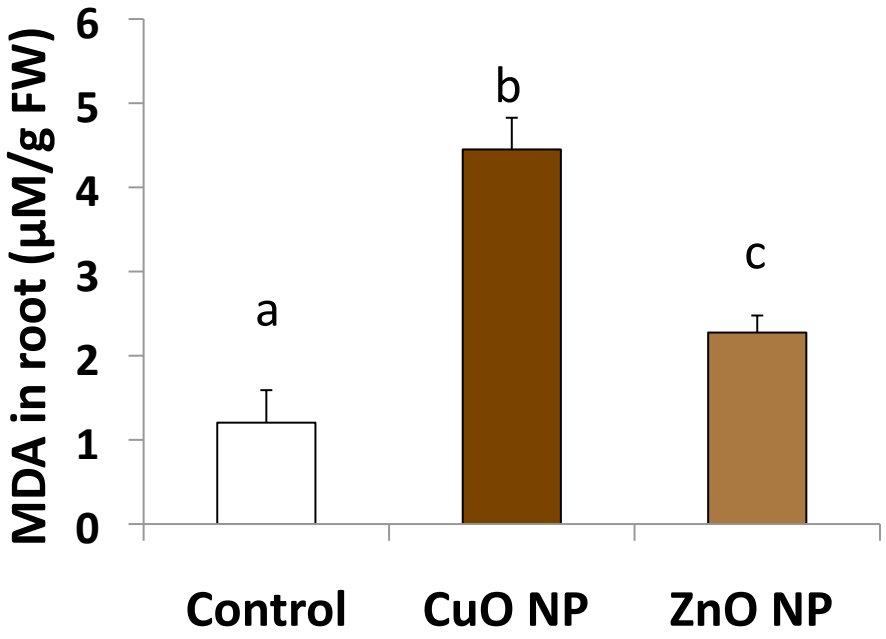



**Siderophore production:**
- Repressed by Fe (expected)
- Repressed by CuO NPs
- Stimulated by ZnO NPs!

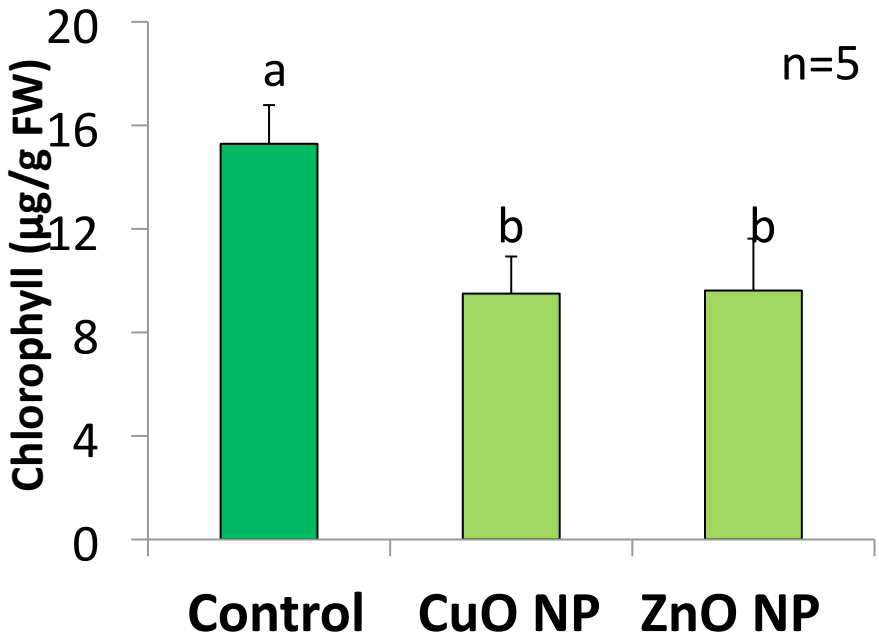
# NP Induced ROS Generation in Wheat

Lipid membrane peroxidation



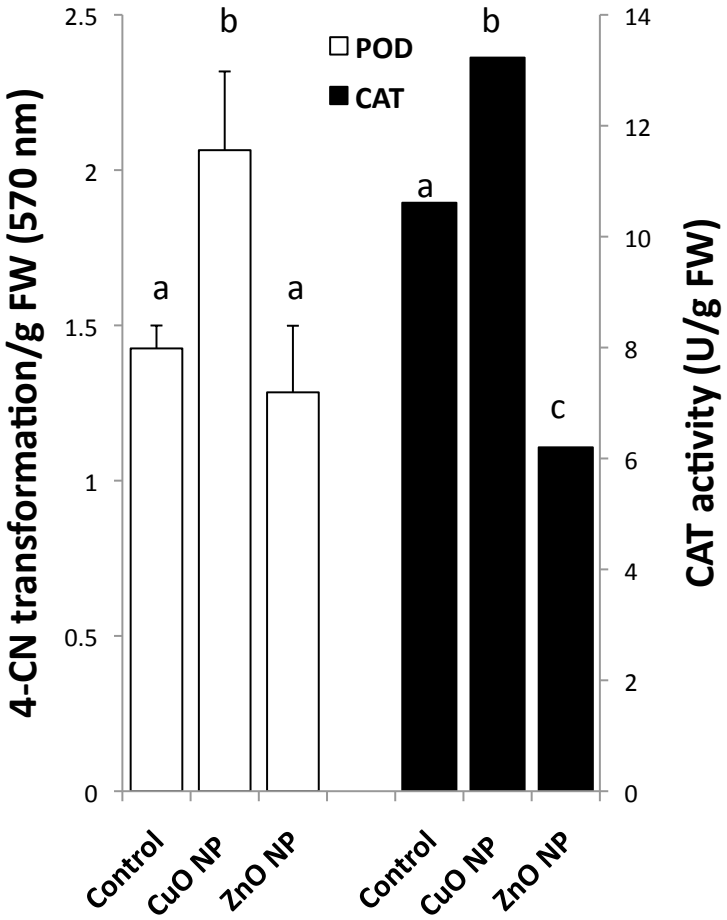

MDA = malondialdehyde

Loss in chlorophyll pigmentation

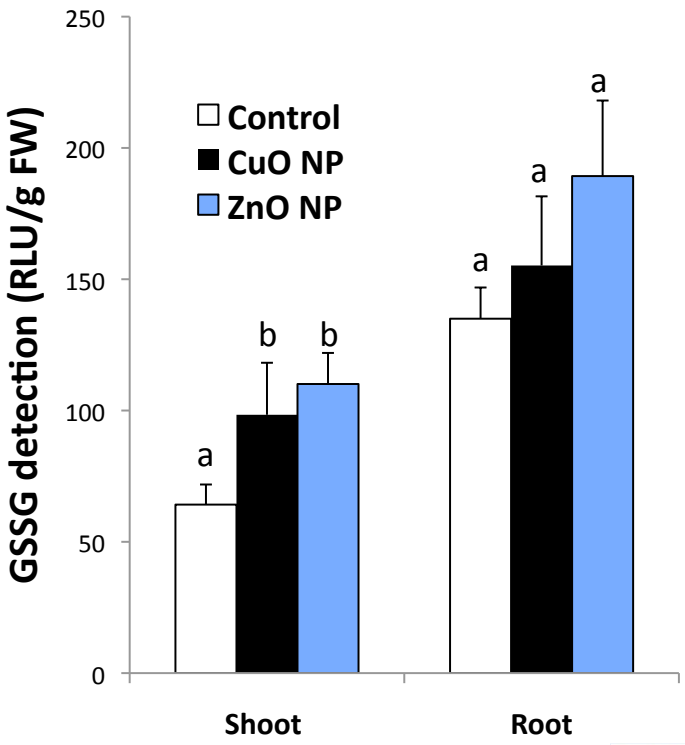


# CuO & ZnO NPs Elicit Antioxidative Stress Responses

Peroxidase & catalase

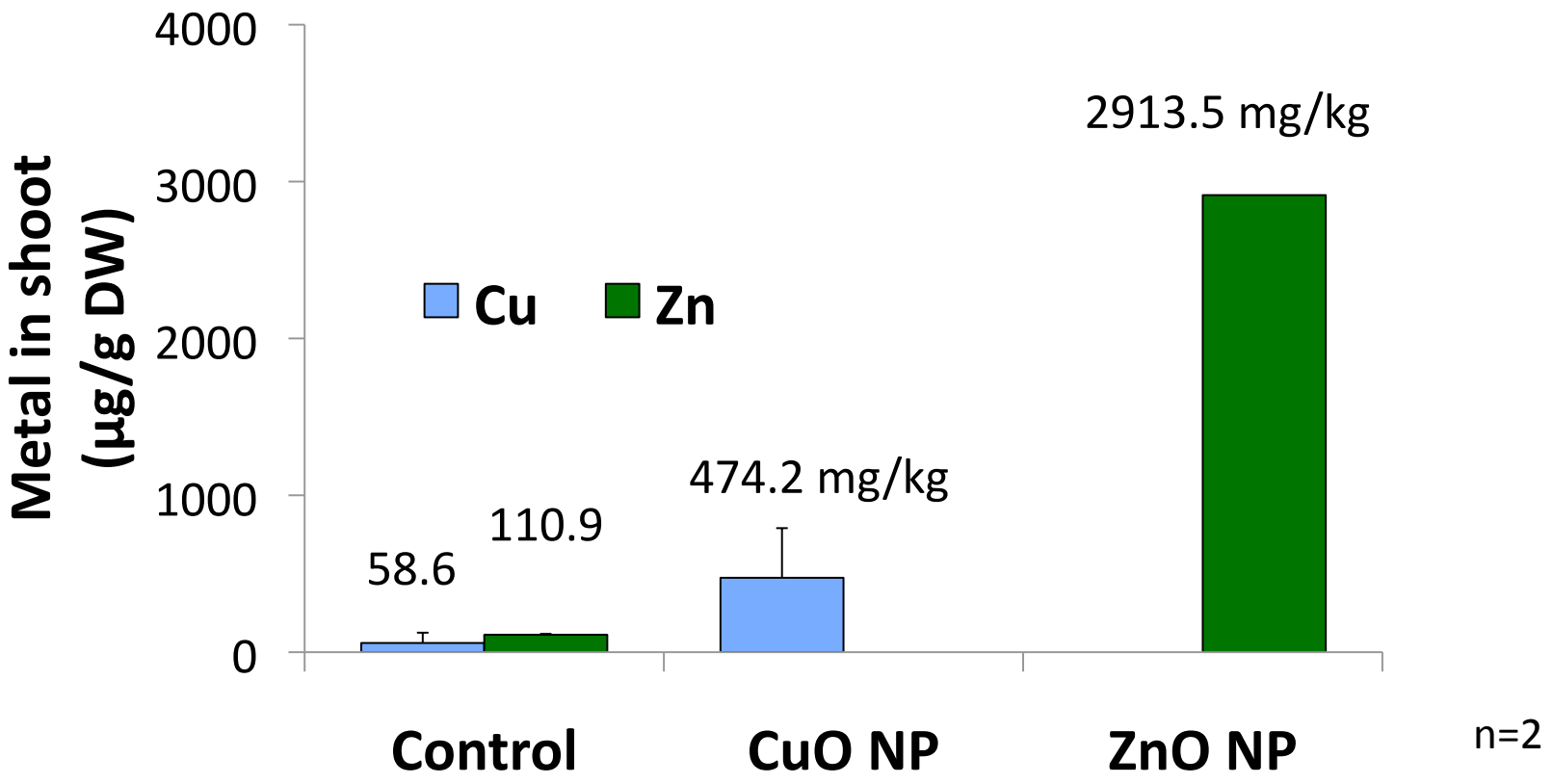



Oxidized glutathione (GSSG)




n=5

# ICP-MS Analysis of Metal Accumulation




n=2

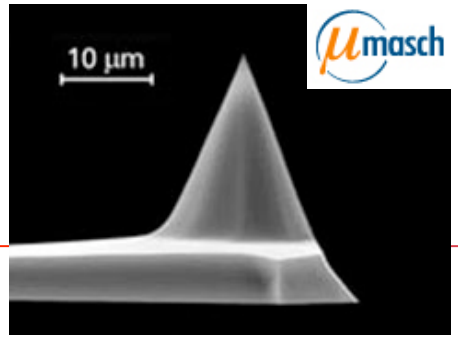
Wheat grown in sand amended with 500 mg NP / kg sand

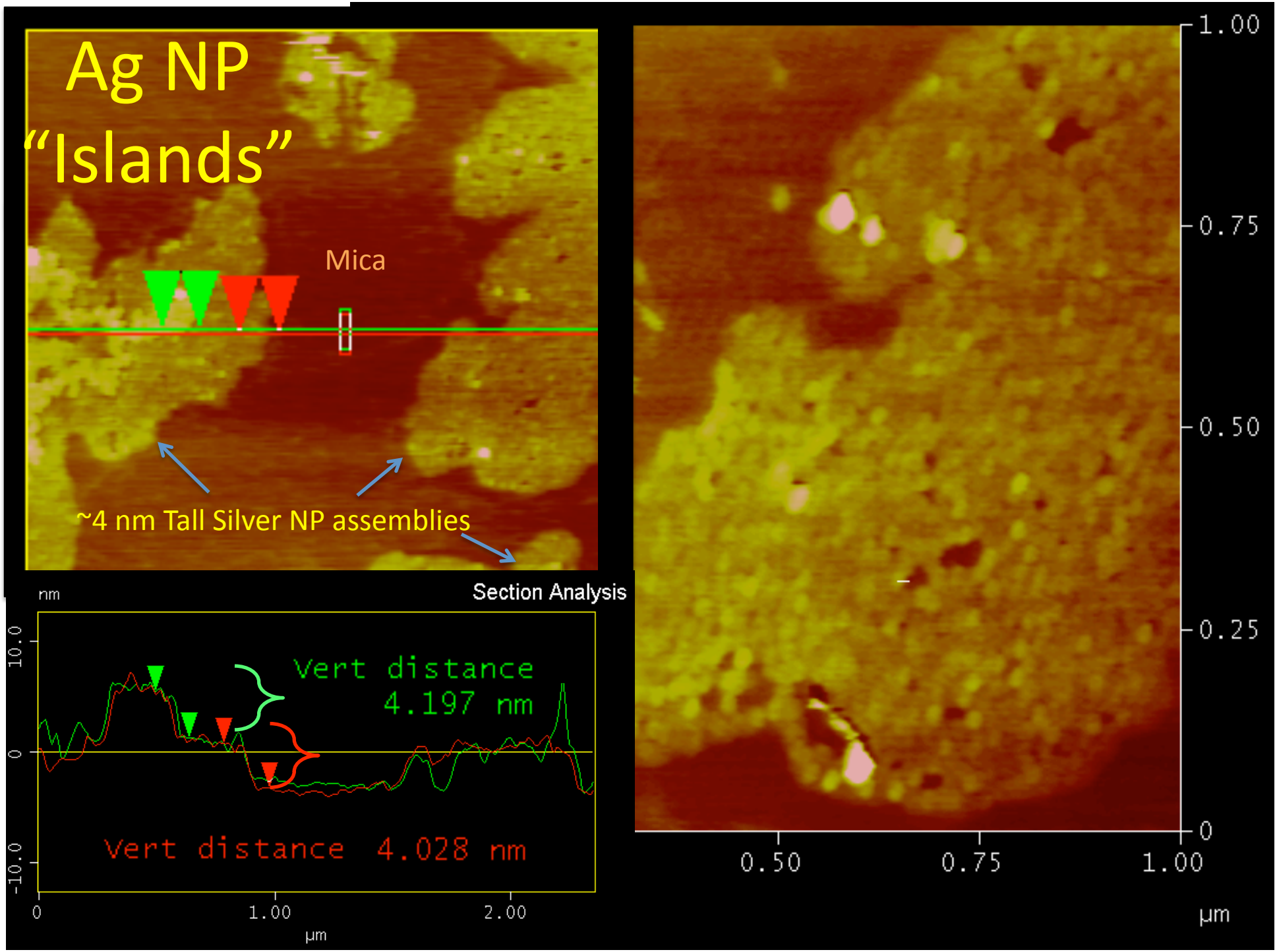
# NP Characterization

- AFM
- FFF

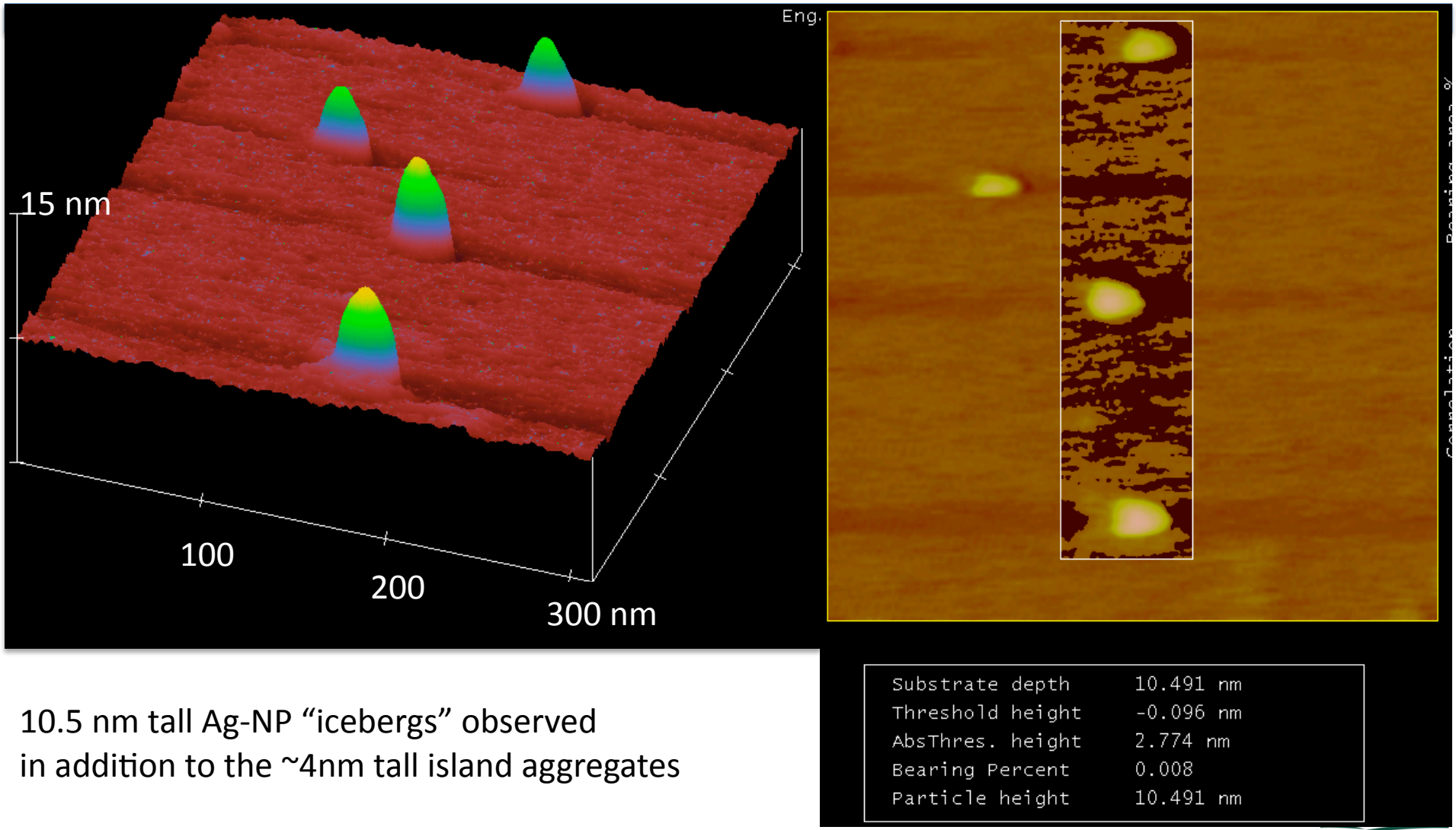
# Atomic Force Microscopy

- Established, fast, inexpensive technique for characterizing NP size / polydispersity
- Method: Evaporate NP solutions on atomically flat mica; contact mode imaging
- Lateral resolution limited by tip radius of curvature (~10 nm). Instrument sensitive to Å differences in z-scale (height)




Ag NP
"Islands"
Mica
~4 nm Tall Silver NP assemblies
Section Analysis
nm
10.0
0
-10.0
Vert distance
4.197 nm
Vert distance 4.028 nm
0
1.00
2.00
µm
1.00
0.75
0.50
0.25
0
0.50
0.75
1.00
µm

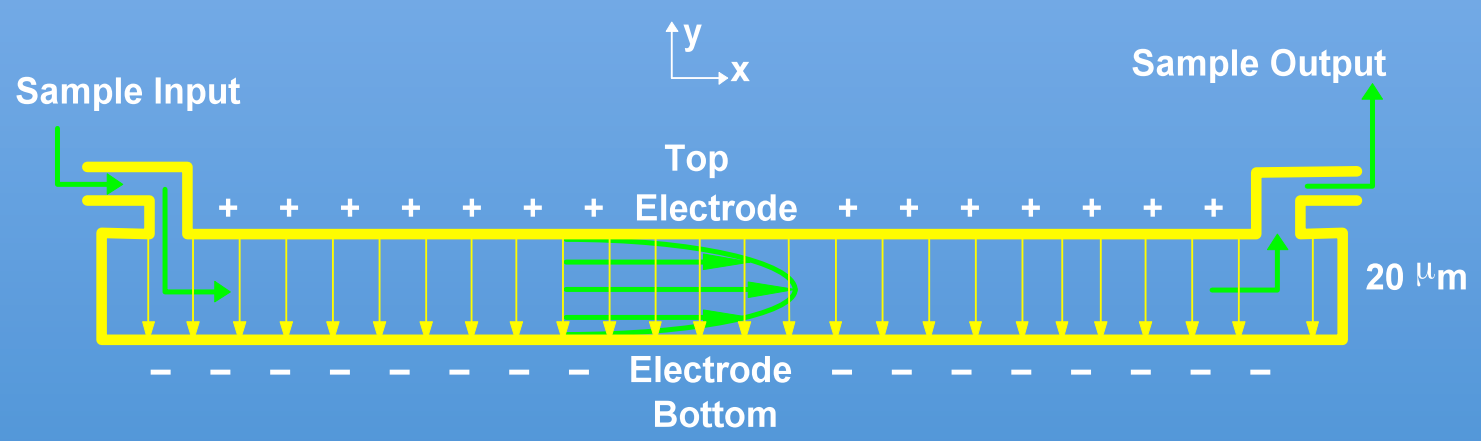
# Ag NPs: 10.5 nm “Icebergs”



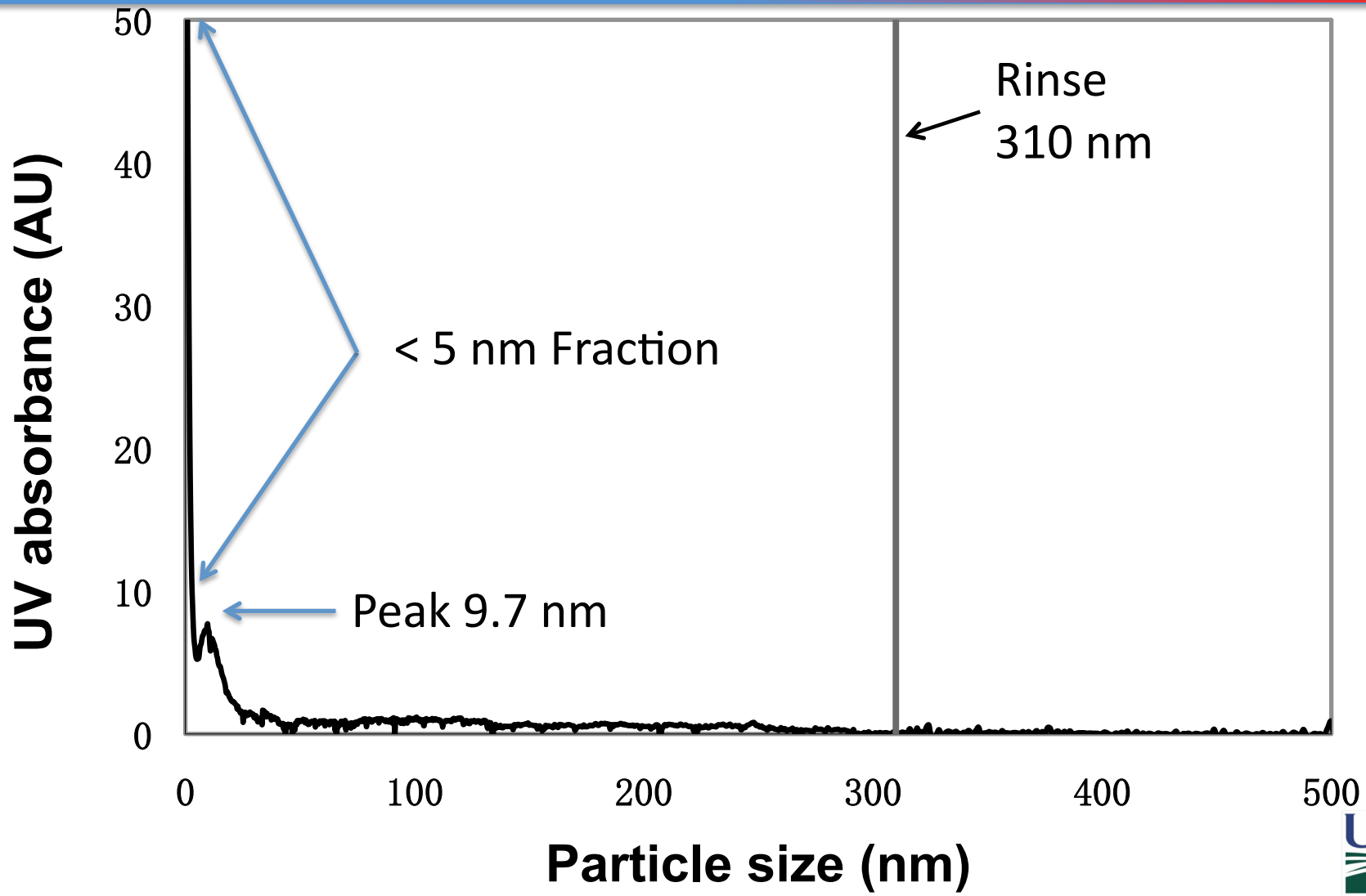

10.5 nm tall Ag-NP “icebergs” observed
in addition to the ~4nm tall island aggregates

# Field Flow Fractionation

- Particle separation technique based on size and/or electrophoretic mobility
- Uses field perpendicular to flow direction
- Field forces particles towards wall
- Relies on laminar flow profile to perform separation
- Distance from wall determines velocity through channel for particles of the same size



# FFF Analysis of Ag NPs



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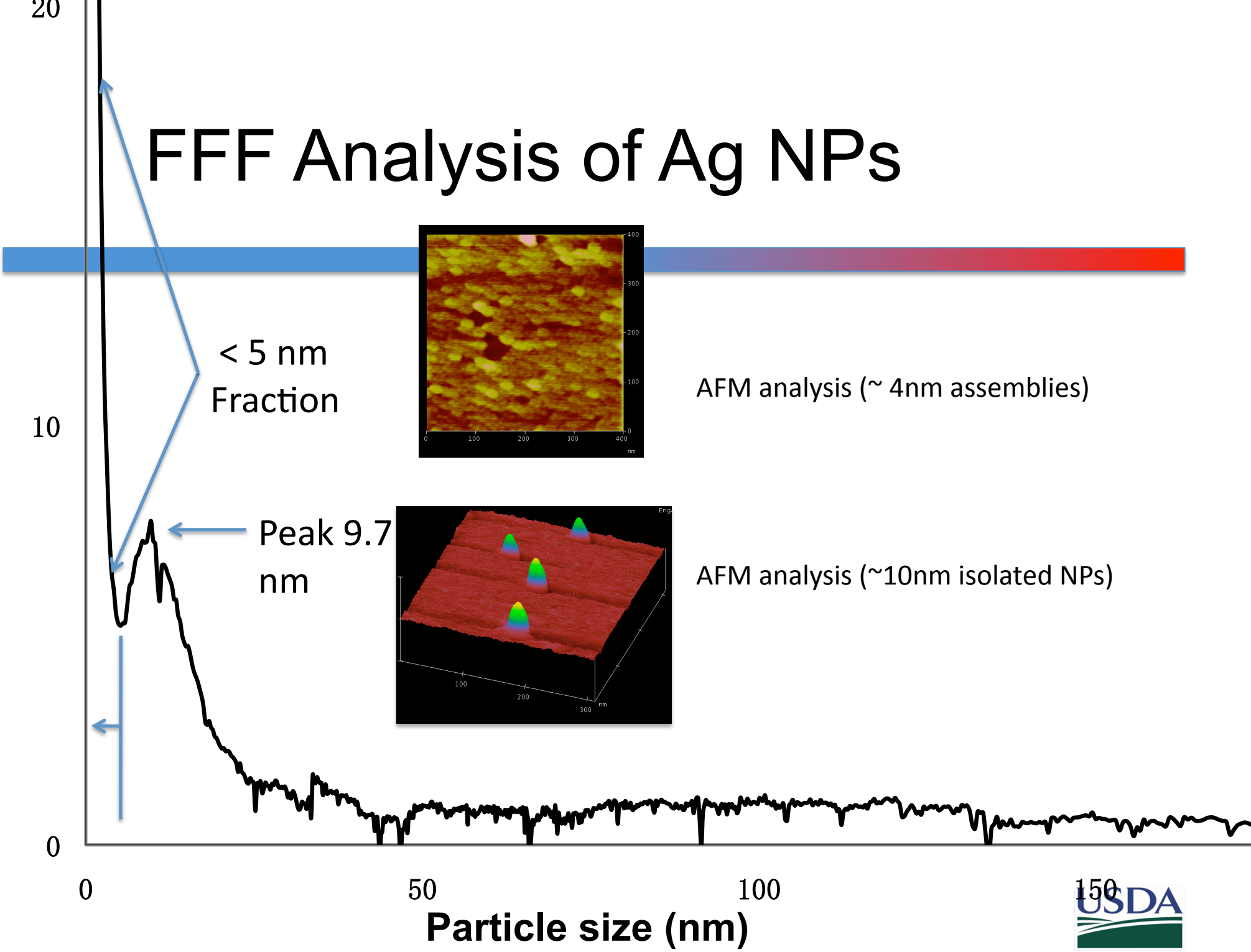
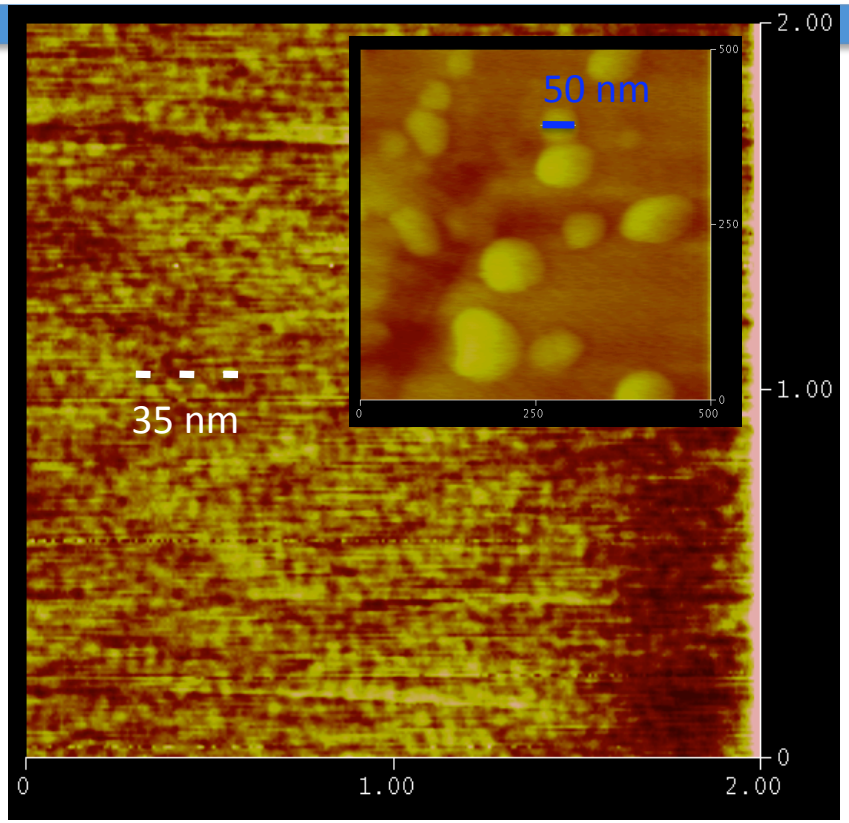
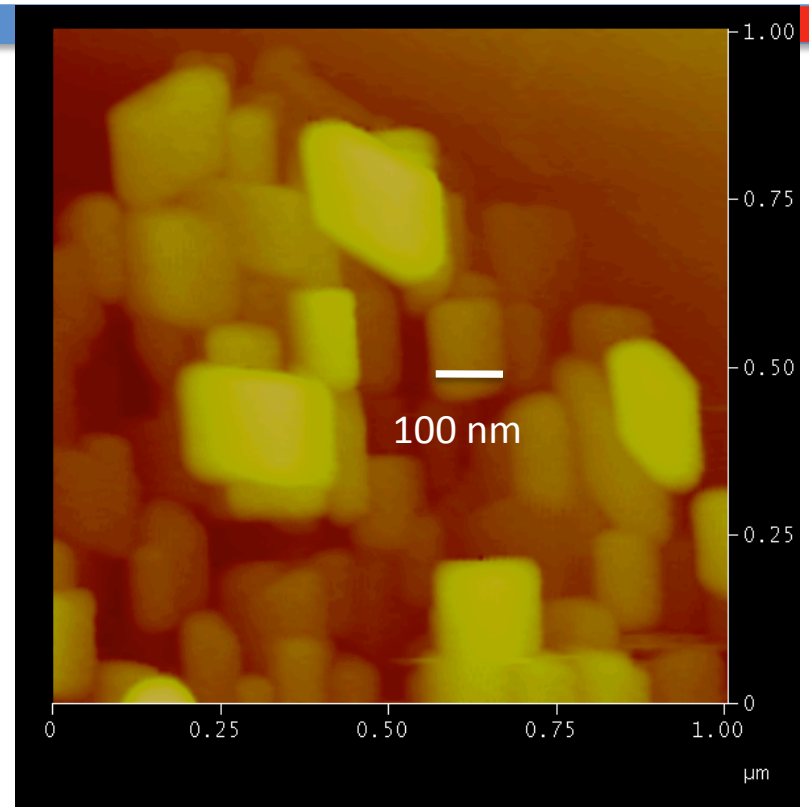


# CuO, ZnO NP Characterization

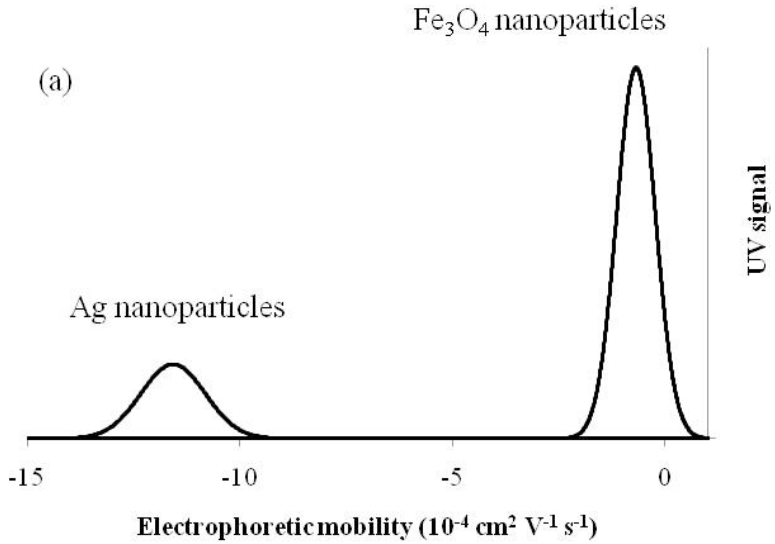


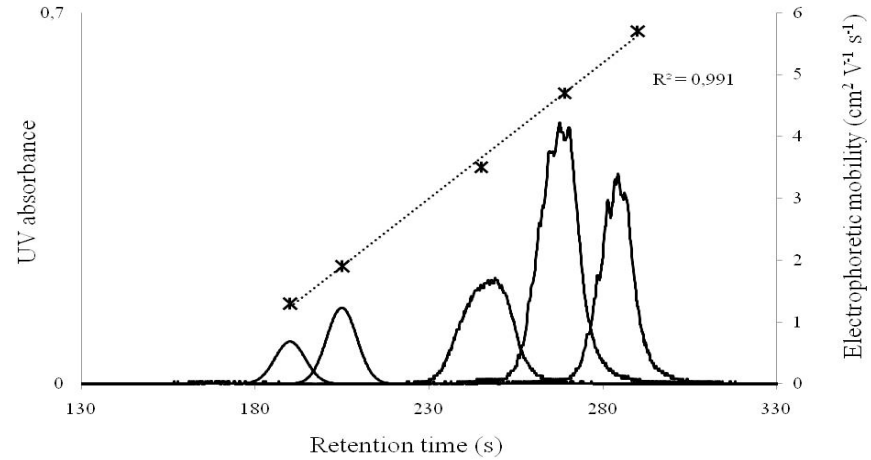



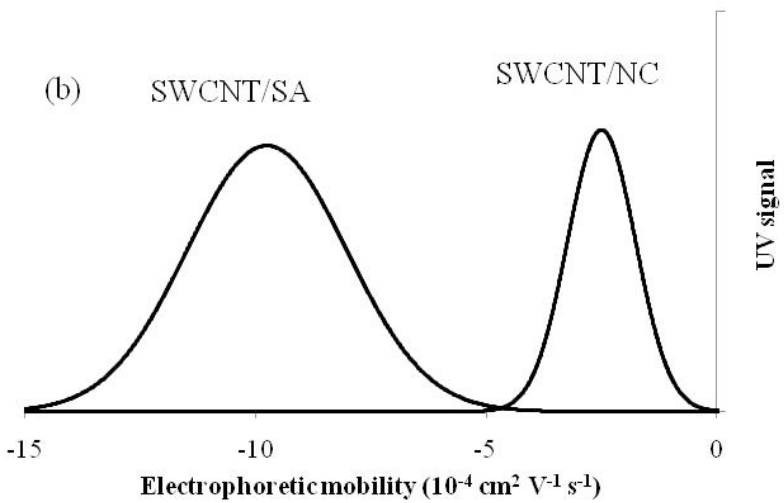

CuO: NP assemblies ~ 35 nm
CuO isolated NPs 35 – 90 nm

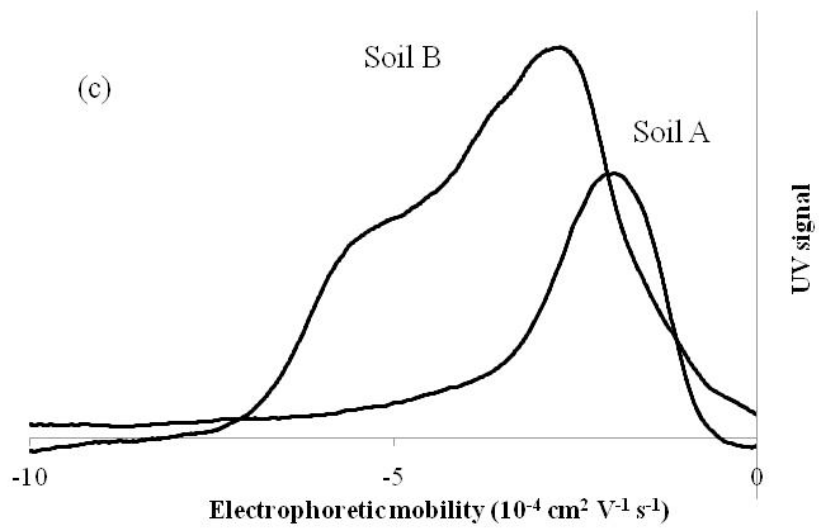
ZnO: Crystalline NPs polydisperse

# Applying FFF to Other NP Systems



# As Requested: Project Key Points

- Is project on target?
  - *Lux* biosensor studies complete
  - Modes of NP action on bacteria and plants continuing
  - NP characterization methods continuing
- What are new findings and achievements since last year?
  - 2009: NP toxicity vs. ions demonstrated using bioluminescent soil microbe KT2440 (question of ion presence in NP solutions)
  - 2010: Key points
    - Assays identifying NP generated reactive oxygen species (ROS) causing toxicity to bacteria and altered plant growth
    - Bacterial EPS demonstrated to protect against NPs and ions
    - Characterization of as-manufactured NPs: AFM, FFF, ICP-MS

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# Project Key Points (cont'd)

- What are the significant contributions to:
  - Nanoscale science, engineering and technology
    - Validated NP characterization methods: FFF, ICP-MS, AFM for Ag, CuO, ZnO, and Be (model NP—and toxic)
    - FFF versatility for a range of NP systems demonstrated
  - Agriculture and food systems?
    - ZnO and CuO NPs demonstrated to differentially regulate two important bacterial metabolites (biofertilizers): Indole Acetic Acid (IAA) and the Siderophore: Pyoverdine (PVD)
- Issues to address in the remaining part of project?
    - FFF: incorporating a multiangle light scattering detector in parallel with UV and ICP-MS detectors to help distinguish NP from ions in the fractionation peaks

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# Acknowledgments


- Funding:
  - USDA-CSREES 2009-35603-05037
  - Utah Agricultural Experiment Station
- Dr. Christian Dimpkpa, USU
- Dr. Srinivas Merugu, U of U
- Alyssa Calder-Anderson (USU undergraduate scholar)
- Wenjie Huang, U of U
- Moon Jiun-Ngooi (USU undergraduate scholar)
- Dr. Jixun Zhan, USU for HPLC assistance
- Publicity: http://www.sciencedaily.com/releases/2009/03/090326162747.htm

# The Prime Movers Behind The Work

Christian, Alyssa, Moon

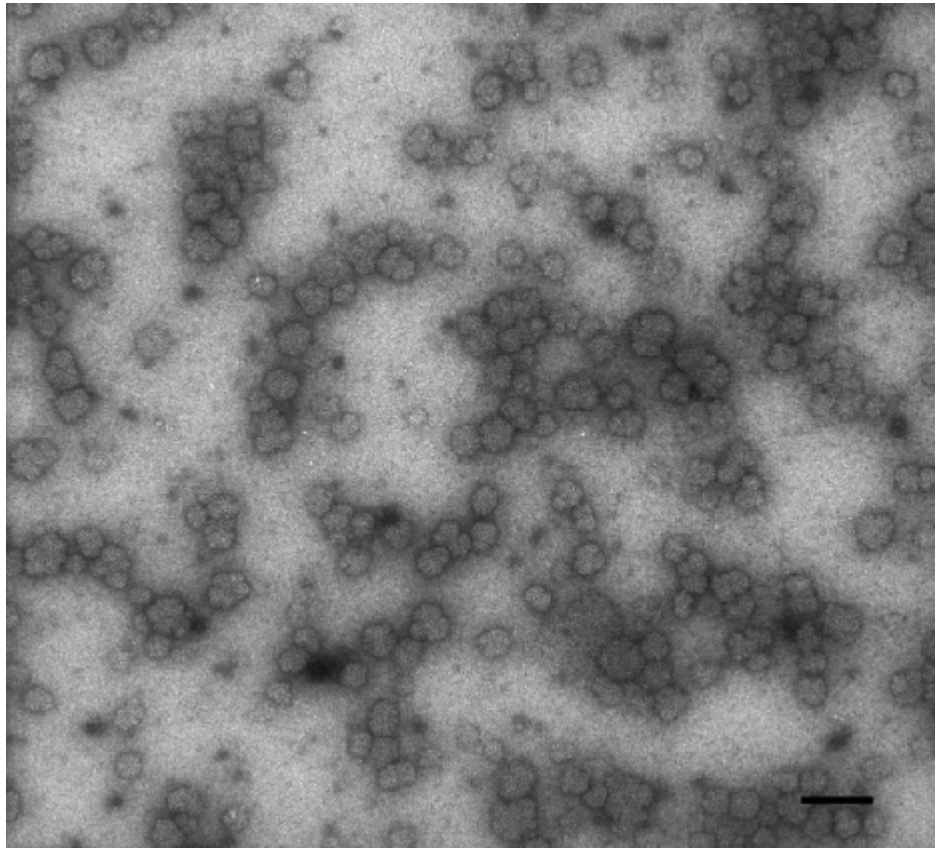
# Phytoglycogen Dendrimer as Nano-carrier For Antibacterial Peptide Loading and Release

- USDA-NRI, Nanoscale Science and Engineering for Agriculture and Food Systems
- Proof-of-concept, one-year project with one-year extension, Jan. 1, 2009 – Dec. 31, 2010
- PIs: Yuan Yao (Food Science, Purdue), Arun Bhunia (Food Science, Purdue), Ganesan Narsimhan (Agricultural & Biological Engineering, Purdue)

# Research background

- Methodology has been established to prepare functional carbohydrate nanoparticles from phytoglycogen, a dendrimer-like polysaccharide isolated from mutant corn
- Researches are being conducted on the behaviors of these nanoparticles in simple and complex colloidal systems
- Potential has been identified for food applications
  - Prolonged efficacy of antimicrobial peptides against pathogens
  - Enhanced oxidative stability of polyunsaturated fatty acids

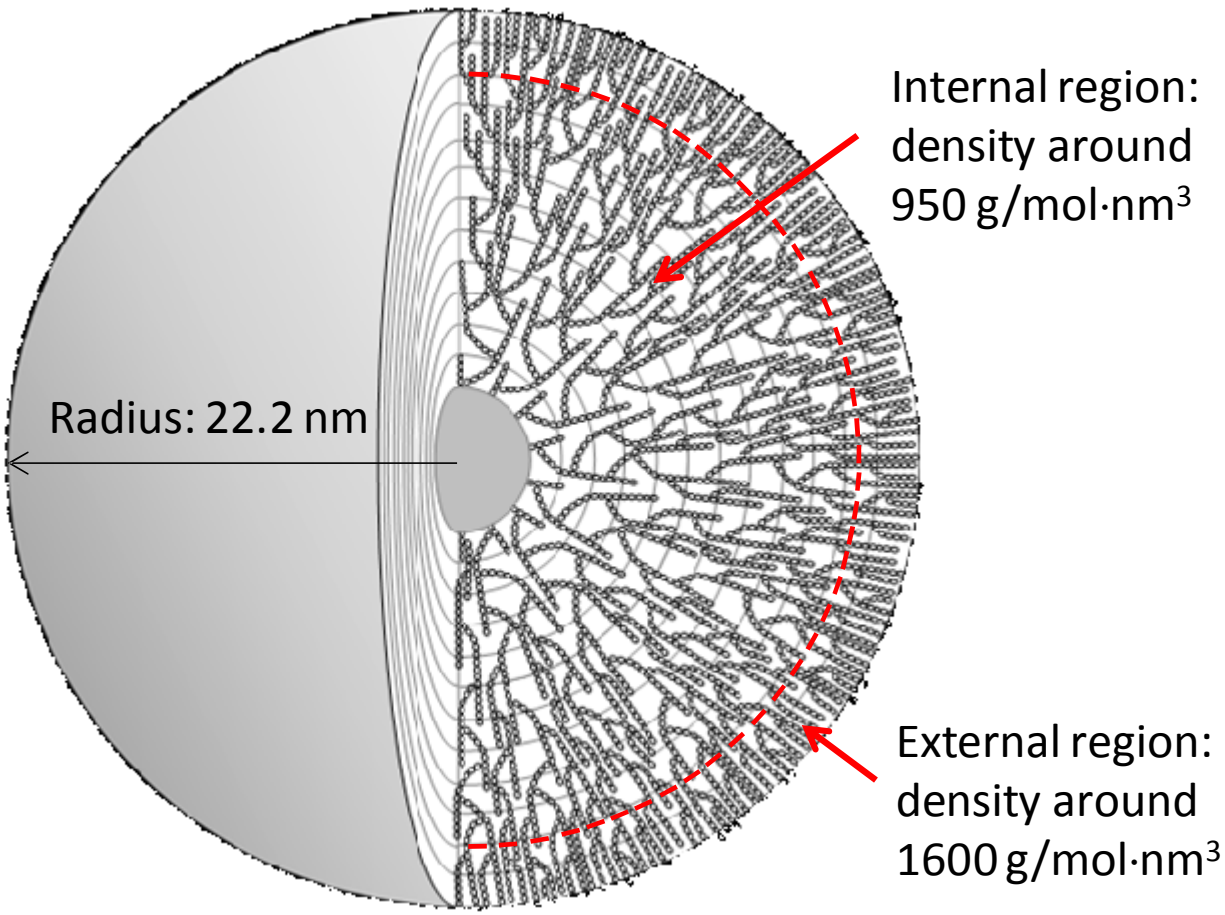
# Phytoglycogen: carbohydrate nanoparticles from plant



**Scale bar: 100 nm**

Huang & Yao, *Carbohydrate Polymers*, 2010

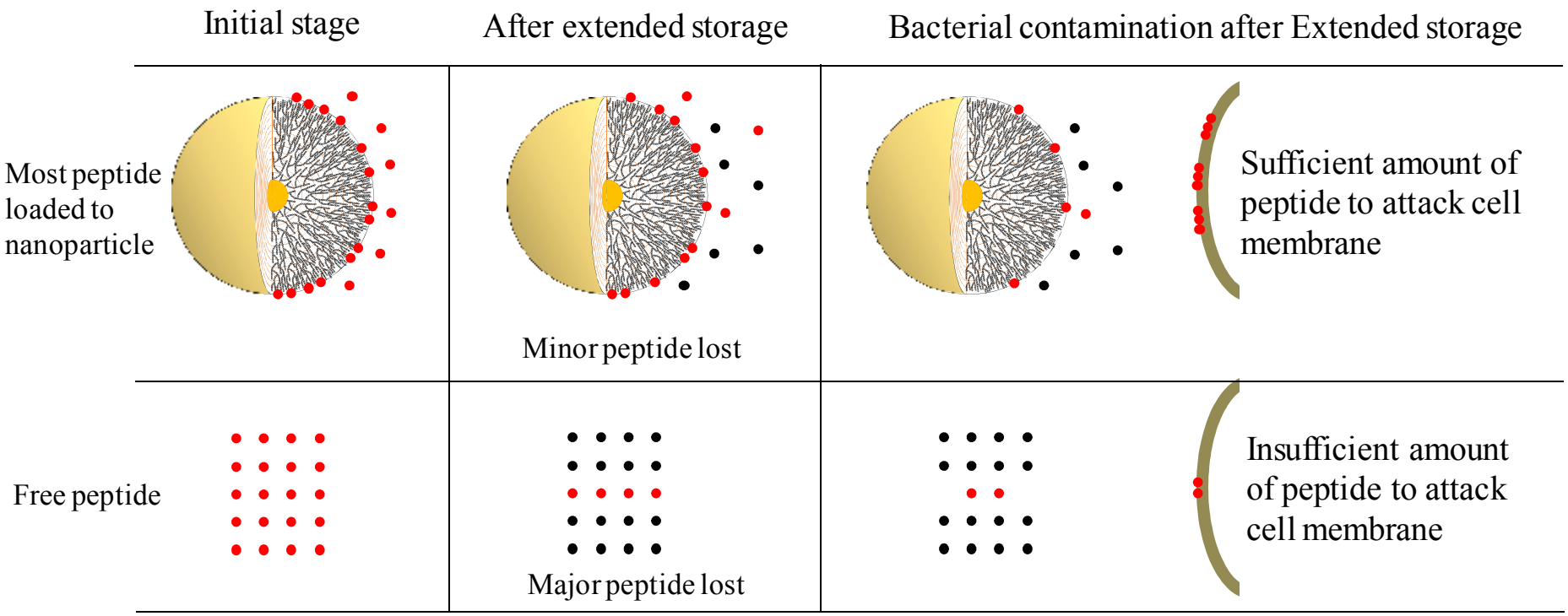
# Phytoglycogen: carbohydrate nanoparticles from plant




Huang & Yao, *Carbohydrate Polymers*, 2010

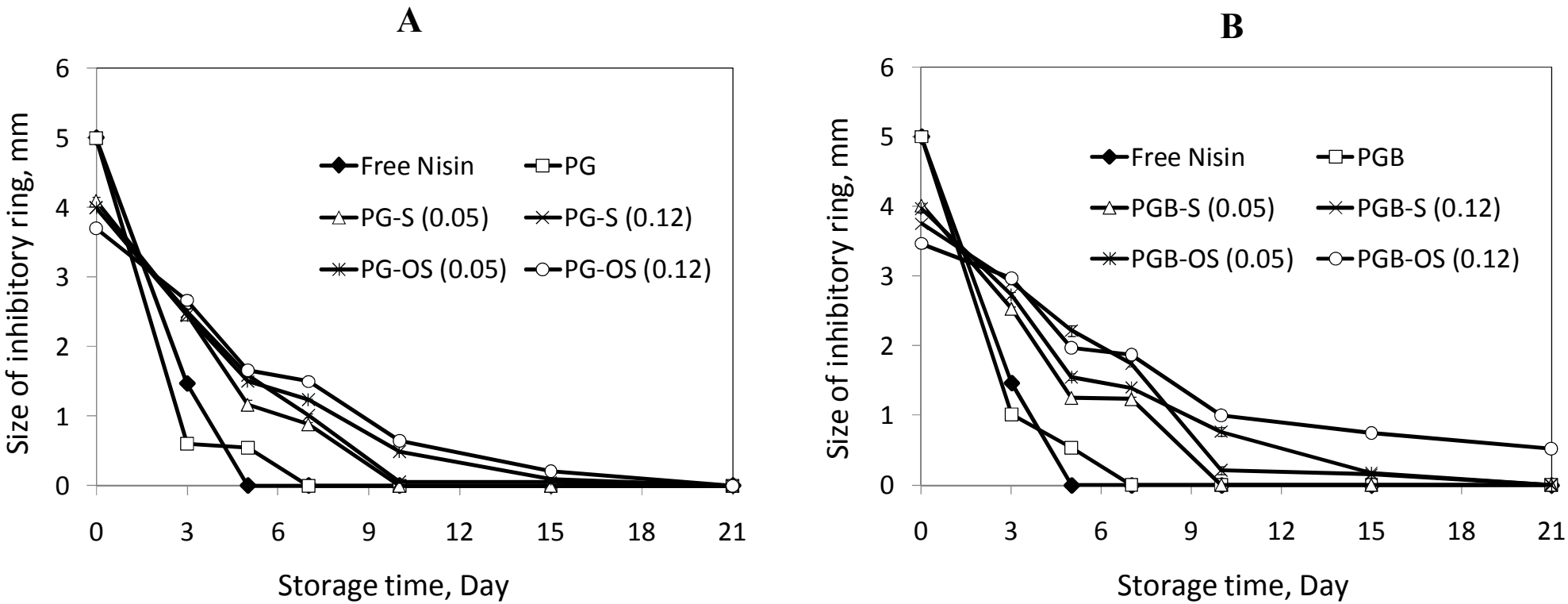
# Strategy-1: PG nanoparticles as delivery device

- Phytoglycogen (PG) subjected to enzymatic and chemical modifications to bring surface charge and hydrophobicity
- PG derivatives tested for prolonged nisin efficacy against *Listeria monocytogenes*
- Results showing PG derivatives very promising for prolonged nisin efficacy




Bi et al., *Journal of Controlled Release*, 2010

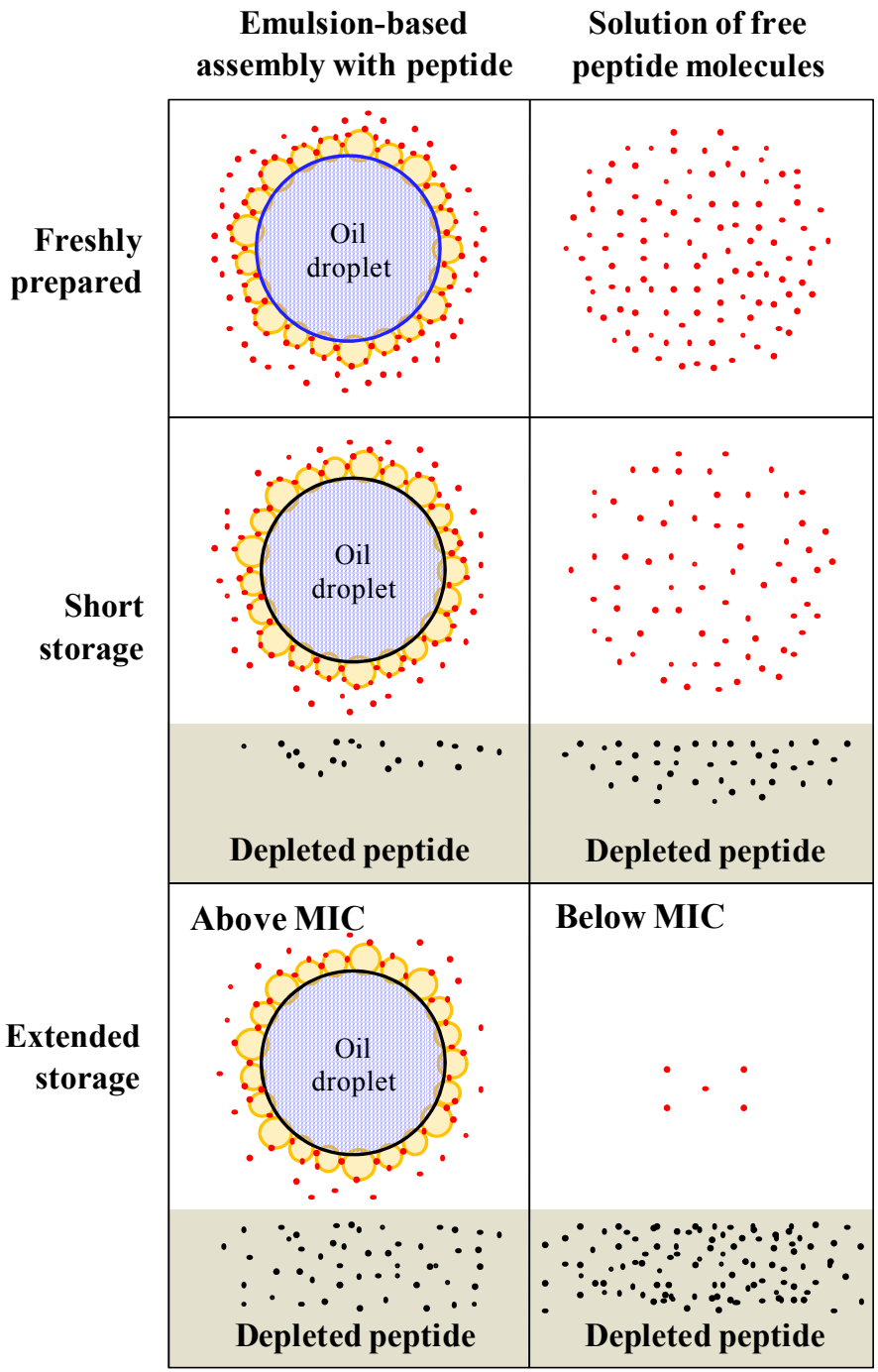
# Strategy-1: PG nanoparticles as delivery device

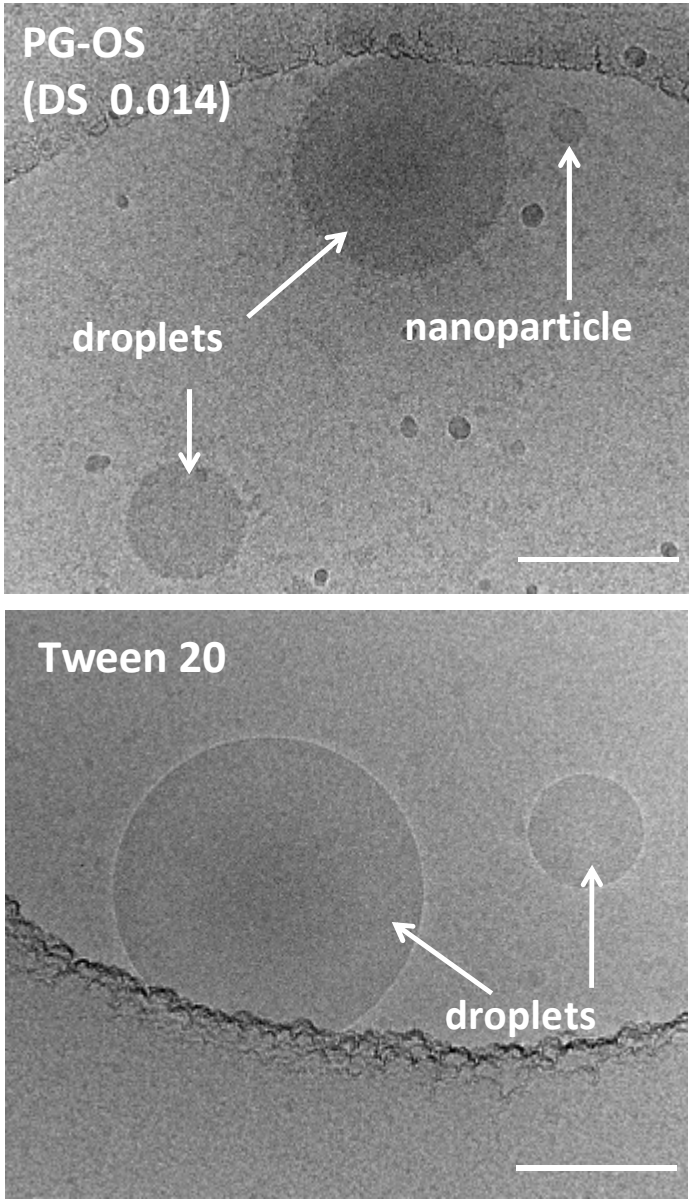



- All PG derivatives showed prolonged retention of nisin activity compared with free nisin
- The longest retention was associated with high degree of substitution, β-amylolysis, and octenyl succinate substitution
- Both electrostatic and hydrophobic interactions were the driving forces of nisin adsorption
- Glucan structure also affected nisin loading and retention

Bi et al., *Journal of Controlled Release*, 2010

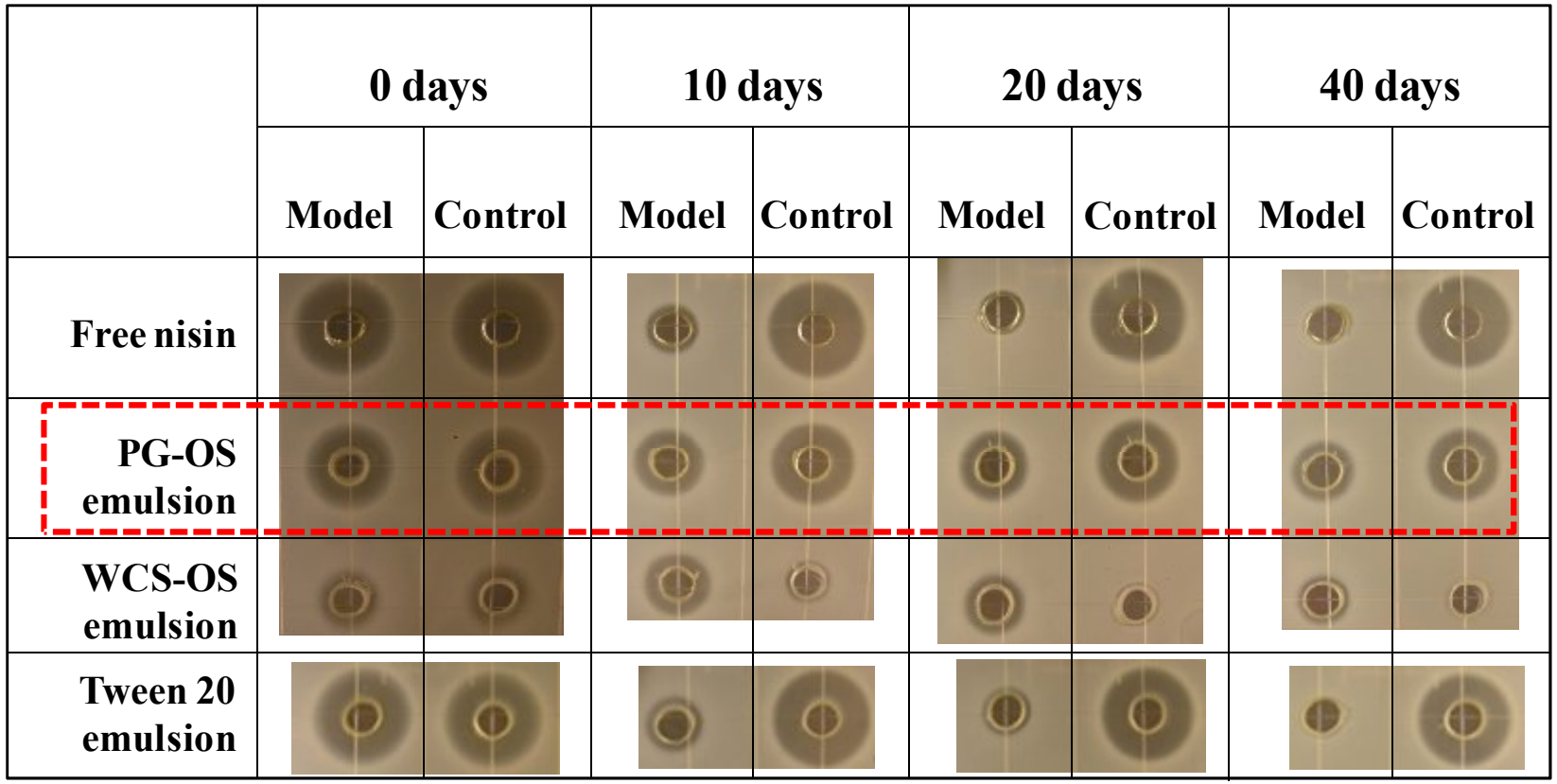
# Strategy-2: PG nanoparticle-stabilized Pickering emulsion




Cryo-TEM, scale bar: 200 nm

# Strategy-2: PG nanoparticle-stabilized Pickering emulsion




- During storage, the anti-listerial activity of nisin-containing PG-OS-stabilized emulsion was much greater than that of nisin solution
- Emulsion stabilized with Tween 20 or modified starch was either ineffective or less effective than the PG-OS emulsion

# Grants, publications, and news release related to phytoglycogen and its functional derivatives

- **NSF grants**
  - Designing carbohydrate nanoparticles for prolonged efficacy of bacteriocin against food pathogens (3-year grant to Yao & Bhunia)
  - Dendrimer-like polysaccharides: structure, modification, and functional colloidal assembly (3-year grant to Yao & Narsimhan)
- **Publications/manuscript**
  - Bi et al. 2010. *J. Control. Release*
  - Huang & Yao. 2010. *Carbohydr. Polym.*
  - Scheffler et al. 2010a. *J. Agric. Food Chem.*
  - Scheffler et al. 2010b. *J. Agric. Food Chem.*
  - Shin et al. 2008. *J. Agric. Food Chem.* (before this grant)
  - Bi et al. 2010. Carbohydrate nanoparticle-mediated colloidal assembly for prolonged efficacy of bacteriocin against food pathogen (*in review*)
- **News release**
  - "Nanoparticle protects oil in foods from oxidation, spoilage", Dec. 2009
  - "Nanoparticle gives antimicrobial ability to fight Listeria longer", Dec., 2010

# Current and future research directions

- Phytoglycogen and glycogen-based nanoparticles, their preparation, properties, and functionalities in various systems, with focus on controlled delivery and modulated digestibility
- Complexation of nisin (and other antimicrobial compounds) with different types of nano-construct, for improved inhibitory efficacy against food pathogens (both Gram Negative and Positive)
- Interactions between carbohydrate nanoparticles and cellular systems, from the perspective of food safety and drug delivery

# Bacteria, Biofilms & Foods: a Nanotechnology-Based Approach

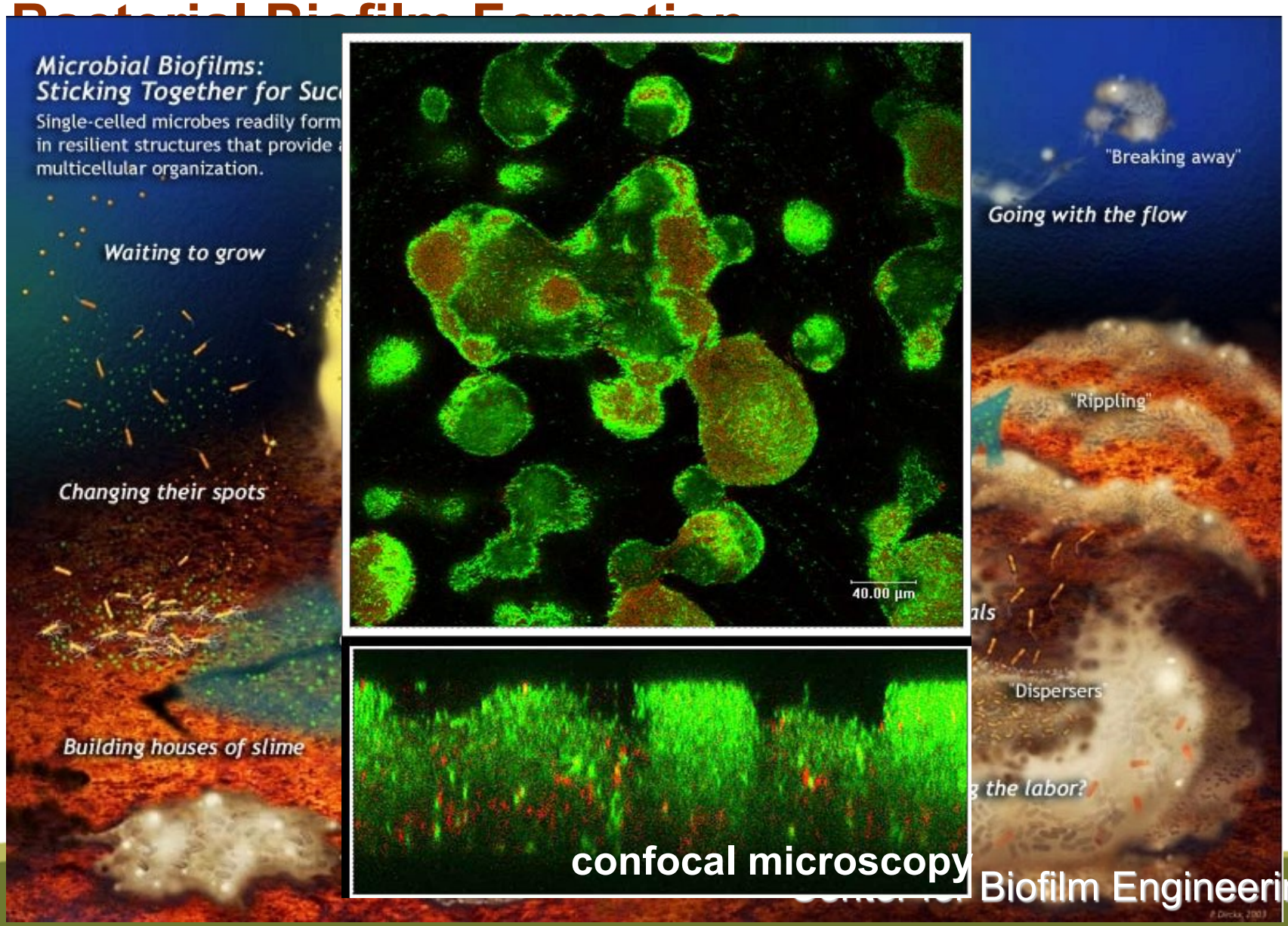
John Dutcher
Professor & Canada Research Chair
University of Guelph

Réseau des aliments et des matériaux d'avant-garde
ADVANCED FOODS & MATERIALS NETWORK
*Inspiration from the ground up | Aux racines de l'inspiration*

# Outline

- multidisciplinary nano-based approach to bacterial biofilms
  - adhesion & survival of cells on surfaces
  - new surface treatments & SOPs to inhibit microbial growth
  - novel cationic peptides that inhibit biofilm formation
- summary & conclusions

# Bacterial Biofilm Formation

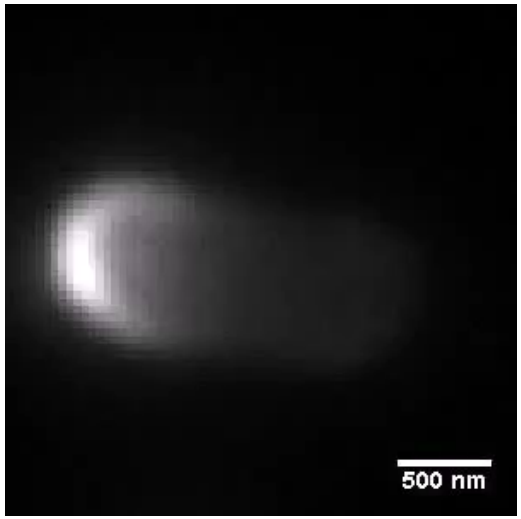


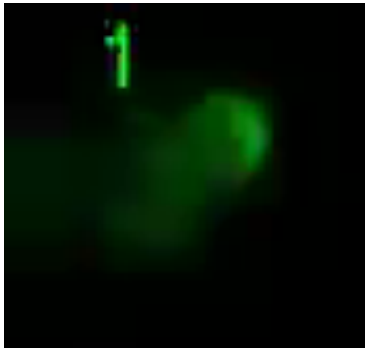
# Goals of Bacteria, Biofilms & Foods Project

- to understand the adhesion of bacteria and the effect of environmental conditions on the survival of bacteria on surfaces
- to develop new surface treatments and standard operating procedures for preventing microbial growth
- to develop novel cationic peptides that inhibit biofilm formation
- to train HQP in a highly multidisciplinary environment

# Individual Cells

- in situ measure of viability of individual bacterial cells
  - min protein oscillations using surface-sensitive fluorescence microscopy
- identified best biological glue (PEI) for cells
- observed high resolution structure in oscillations
- measuring oscillation period in response to stress (antimicrobials, applied pressure, etc.)

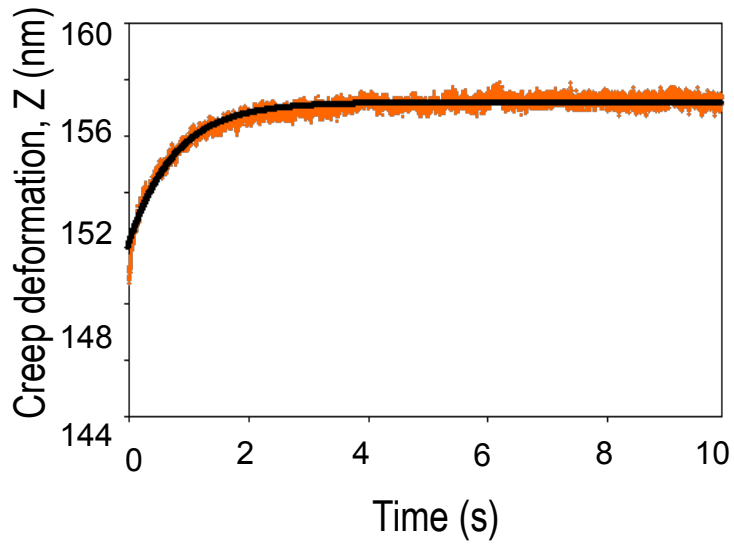


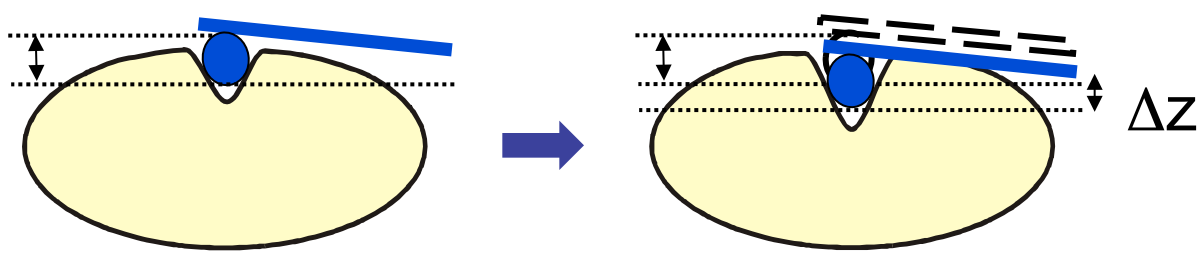




addition of polymyxin B at frame 40

# Individual Cells

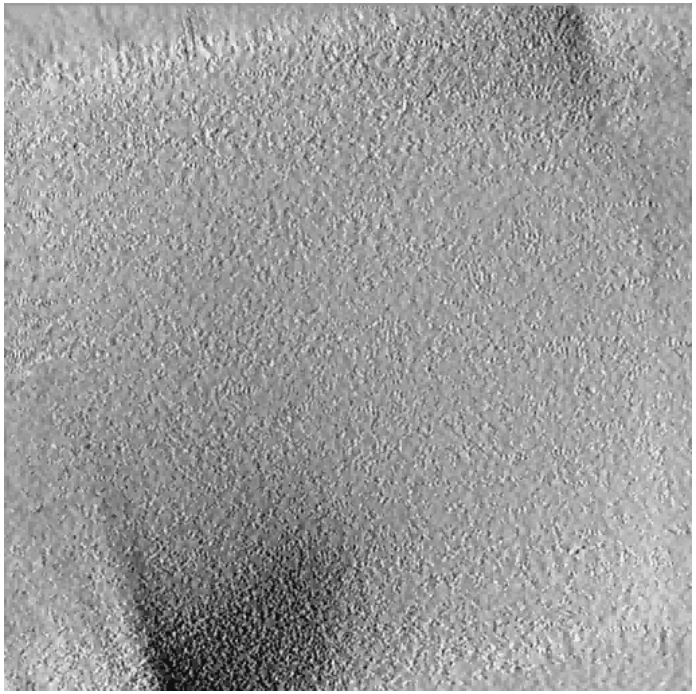
- measurement of mechanical properties of individual cells
  - AFM nano-creep experiment
- time-dependent response to constant applied force
  - Gram-positive vs Gram-negative
  - effect of lipoproteins (stiffness of cell wall)



# Individual Cells

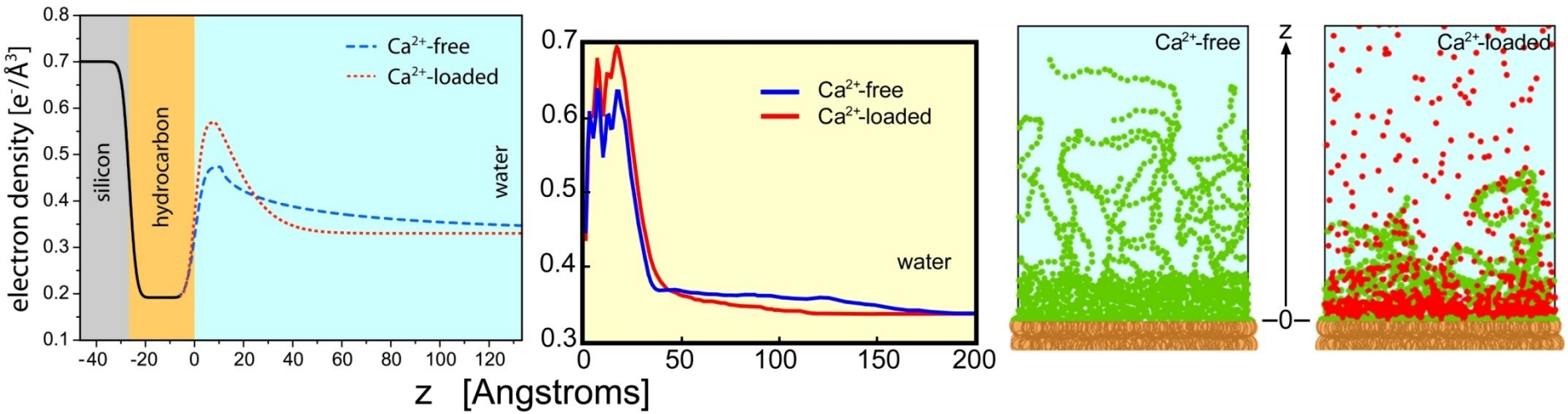
- whole cell electron tomography
  - turning 2D images into 3D images
- plunge freezing of samples
- high resolution sections
  - high resolution 3D images of cell walls (effect of antimicrobials, nanoparticles)



*Caulobacter crescentus*

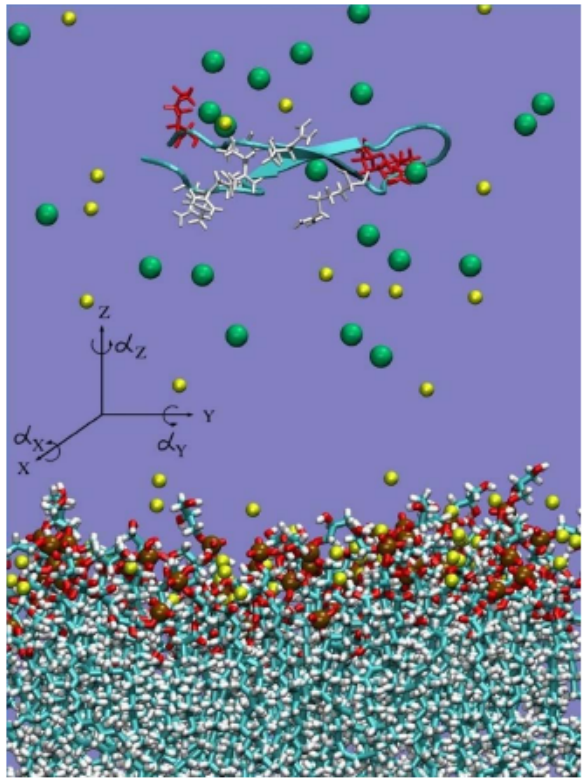
# Individual Cells

- use x-rays and computer simulations to study protamine interacting with lipopolysaccharide monolayers
  - effect of excess $Ca^{2+}$ ions



# Antimicrobial Action

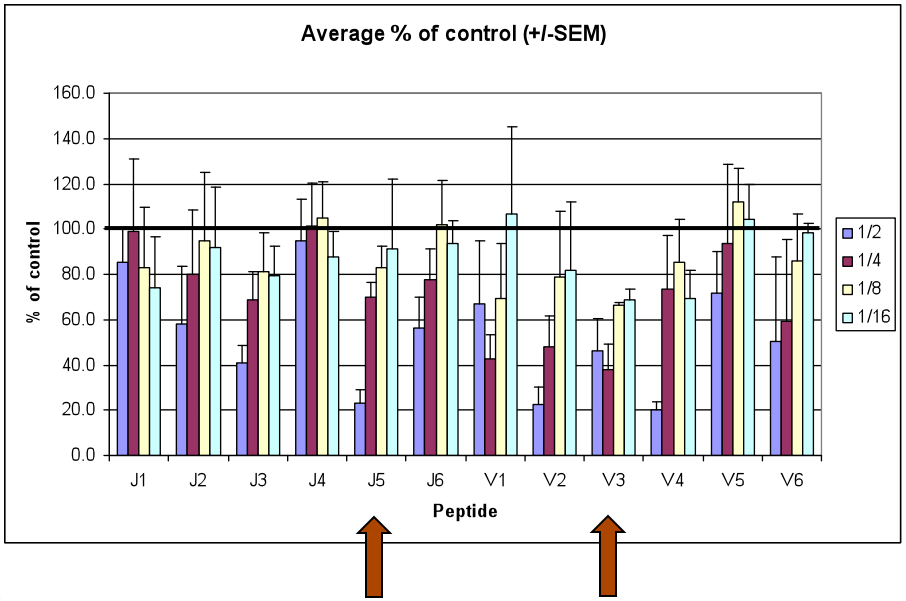
- computer simulations of new approach to measurement of binding energy of peptide molecules to cell surface




GPU based high performance computing cluster

# Anti-Biofilm Action

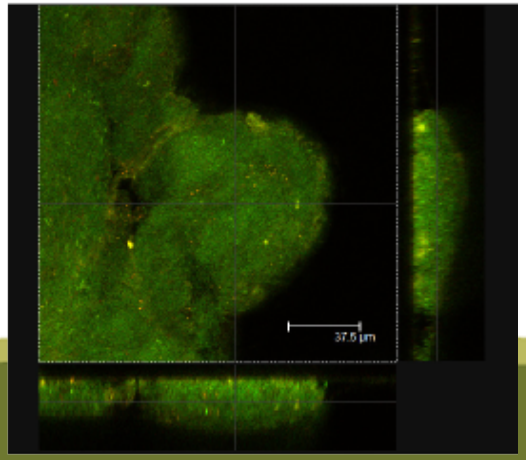
- originally discovered anti-biofilm activity for LL-37 peptide
- screened large number of peptides for activity versus biofilms
  - peptides 7-12 aa long
- identified promising candidate peptides vs. *P. aeruginosa* (Gram-negative)
  - also showed activity vs. *L. monocytogenes* (Gram-positive)




- iteratively designed new peptides
  - some had improved anti-biofilm activity

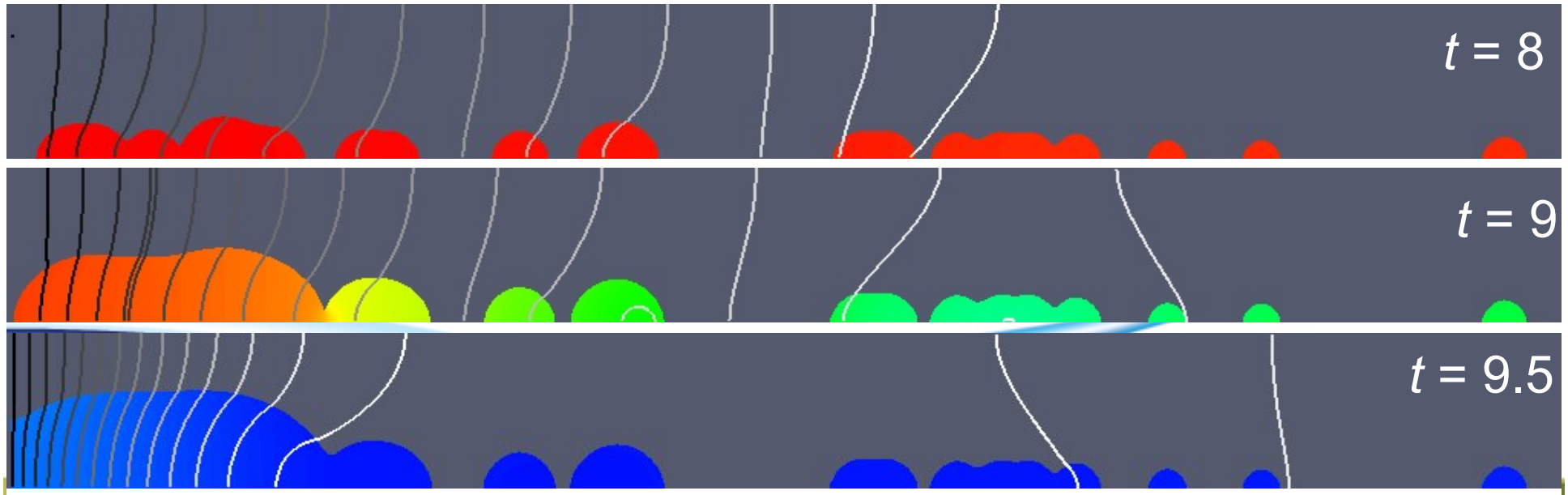
# Biofilms

- measurement of cohesive strength of biofilms
  - AFM-based experiments
- time-dependent response to constant applied force
  - effect of lipopolysaccharide core capping on mechanical properties of *P. aeruginosa* biofilms
  - correlation to changes in biofilm structure measured using confocal microscopy



# Biofilms

- developed mathematical models of iron chelation and quorum sensing in biofilms
  - results support concept of efficiency sensing
  - up/down regulation is a consequence of interplay of mass transfer and quorum effects




flow

## Next Steps

- screen new antimicrobial and anti-biofilm agents
- interaction of nanoparticles with bacterial cells & biofilms
  - delivery of antimicrobial compounds by nanoparticles
- chemical effects and biofilms
  - iron chelation and upregulation
- biofilm control using surface coatings, environmental control & interfering with bacterial structure-function organization
  - microbial food spoilage

# Summary & Conclusions

- nanotechnology-based strategies to study bacteria on surfaces
  - sophisticated nano-techniques to study individual cells & biofilms
    - experiments & computer simulations
    - new antimicrobials & anti-biofilm compounds
    - new surface coatings & SOPs
- big opportunities for nanoscale science & technology with real world benefits

afmnet

Réseau des aliments et des matériaux d'avant-garde
ADVANCED FOODS & MATERIALS NETWORK
*Inspiration from the ground up | Aux racines de l'inspiration*

# Food Micronutrient and Flavor Release in Nanostructured Matrices

Susan Ebeler, Ronald Phillips, Stephanie Dungan
University of California, Davis

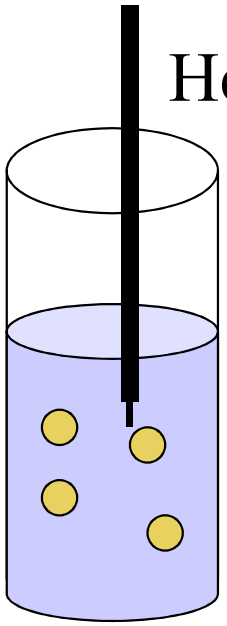
USDA Nanoscale Science and Engineering for Agriculture and Food Systems

Progress Report

September, 2010

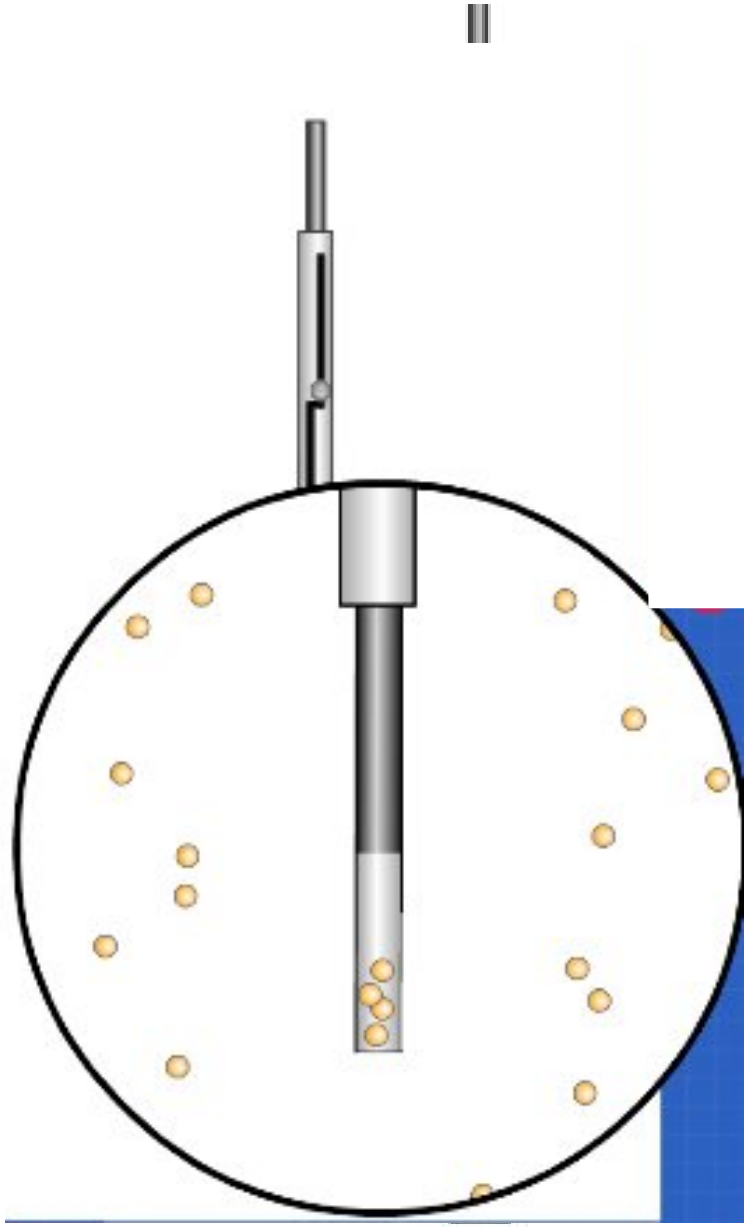
# Solid Phase Microextraction (SPME)-GC/MS

- **Capture hydrophobic solutes in fiber**
  - **Probe gas**
  - **Probe continuous phase**



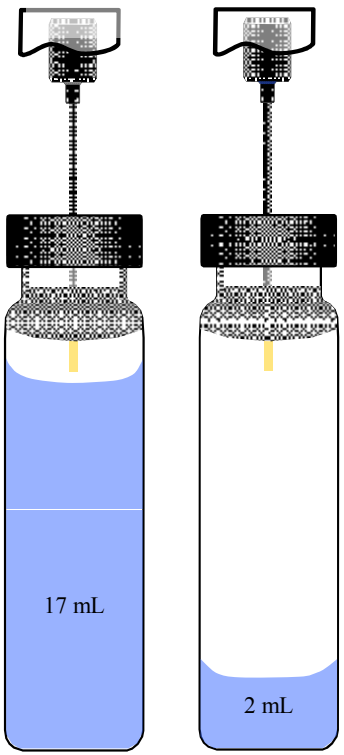
Headspace (HS)

Direct immersion (DI)

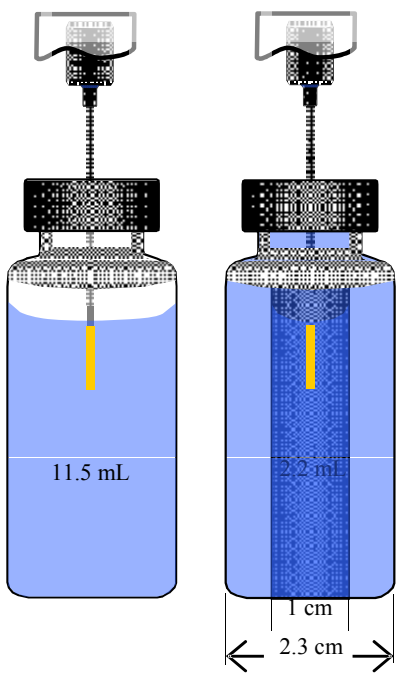


# Probe Vapor or Liquid

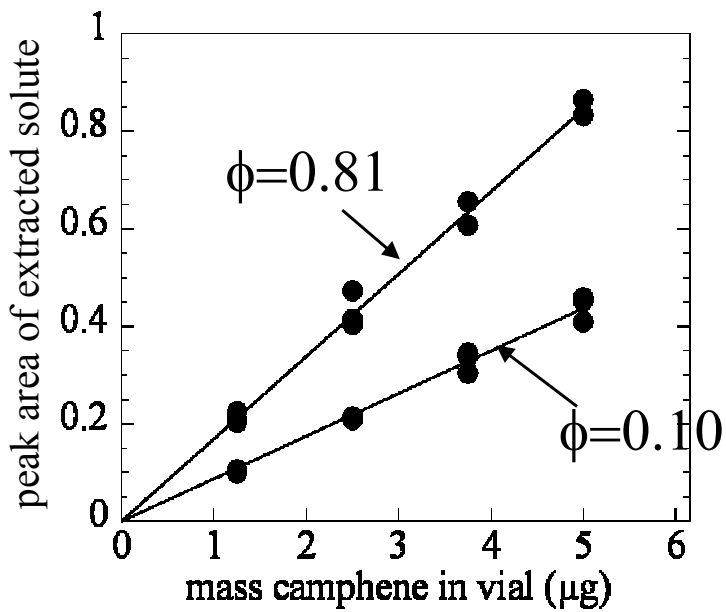
- **Can independently probe vapor phase (headspace) and liquid phase (direct immersion)**




**HS-SPME**

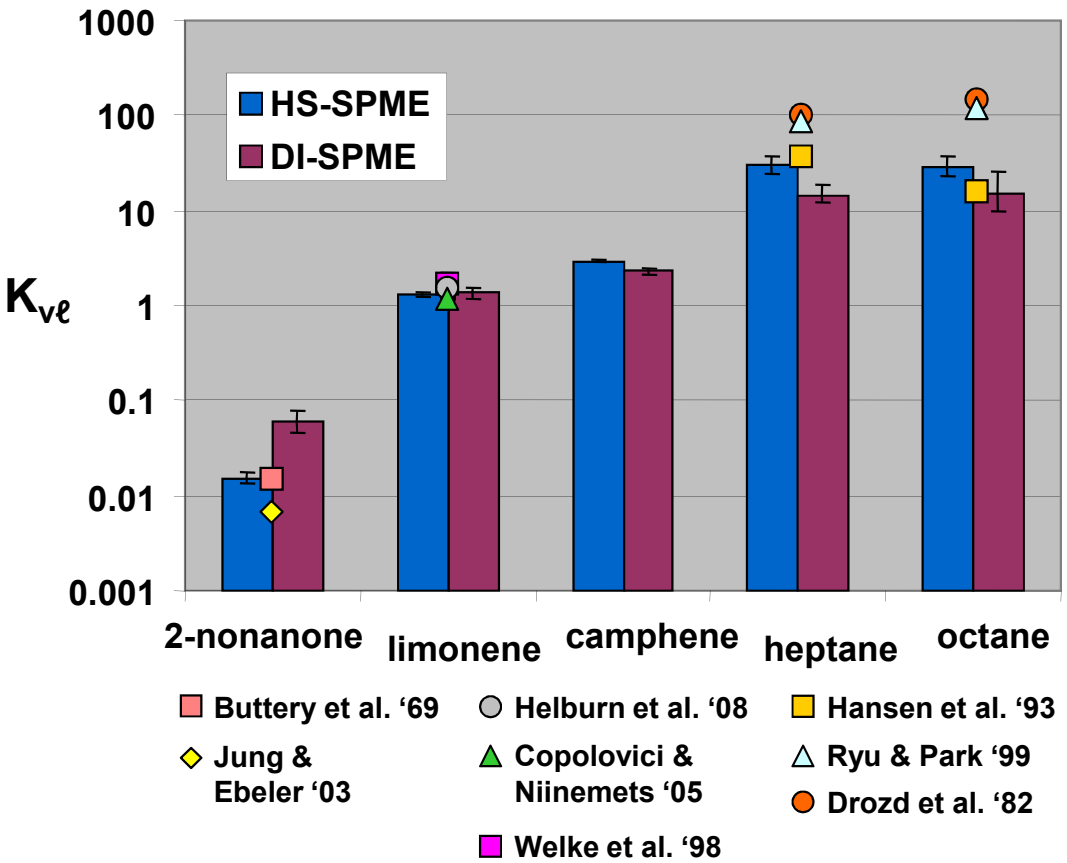



**DI-SPME**




$$\frac{\text{Slope}_1}{\text{Slope}_2} = \frac{V_T^{(1)}}{V_T^{(2)}} \frac{\phi_2 + K_{vl}(1-\phi_2)}{\phi_1 + K_{vl}(1-\phi_1)}$$

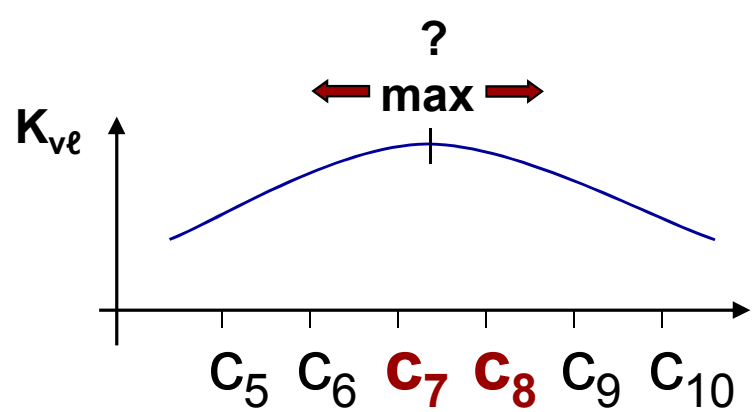
# Extraction from Multiphases



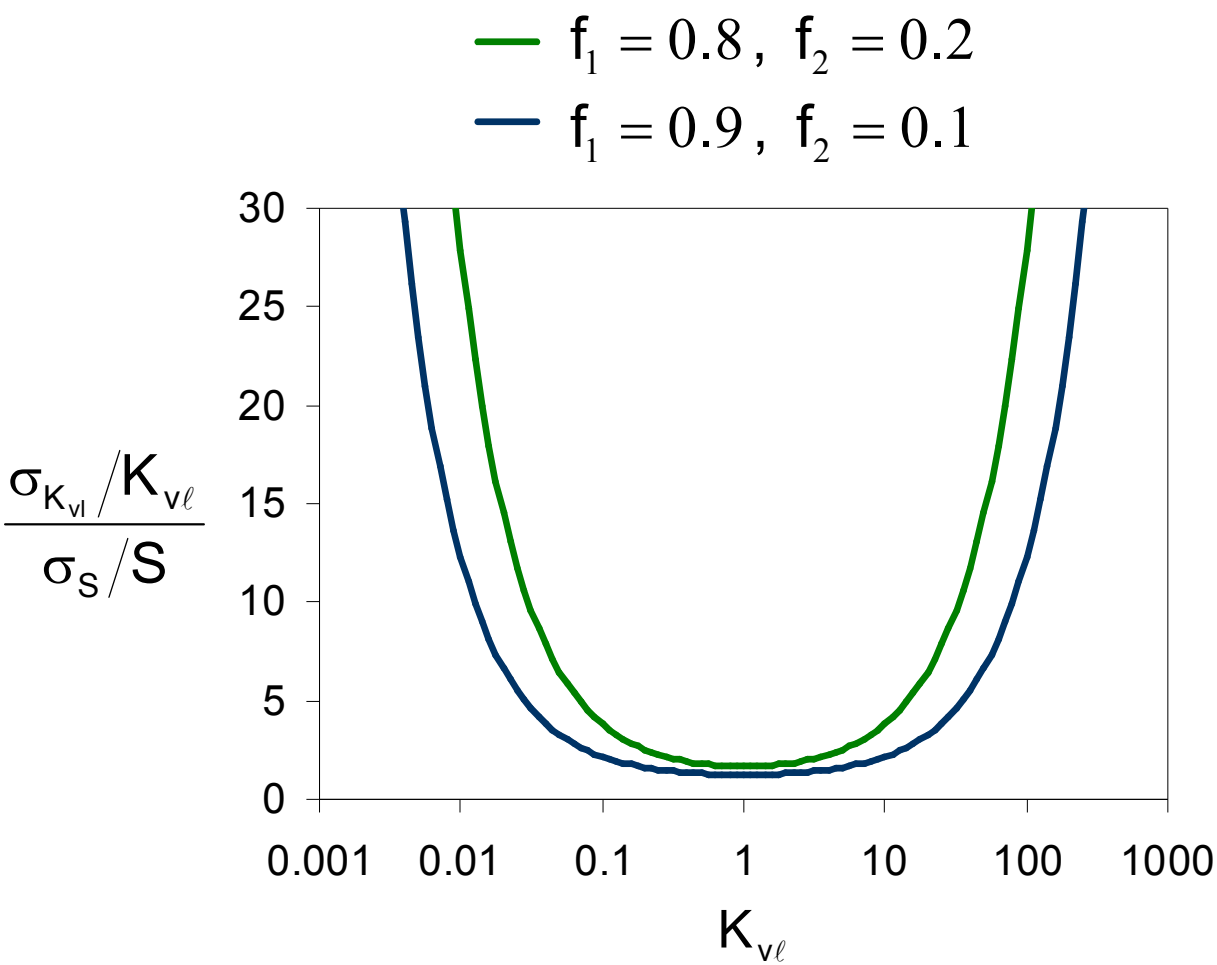

**Agreement over 4 Orders of Magnitude in $K_{v\ell}$**

**Variability increases for low and high $K_{v\ell}$**

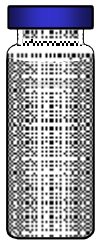
**Heptane & Octane**



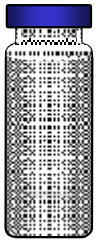
# Error Analysis




- Fill fractions can strongly affect $K_{v\ell}$ precision
- Greater precision for compounds with $K_{v\ell} \approx 1$



Vary n with $f_1 = 0.9$



Vary n with $f_1 = 0.1$

# Partitioning of Limonene w/ Microemulsion

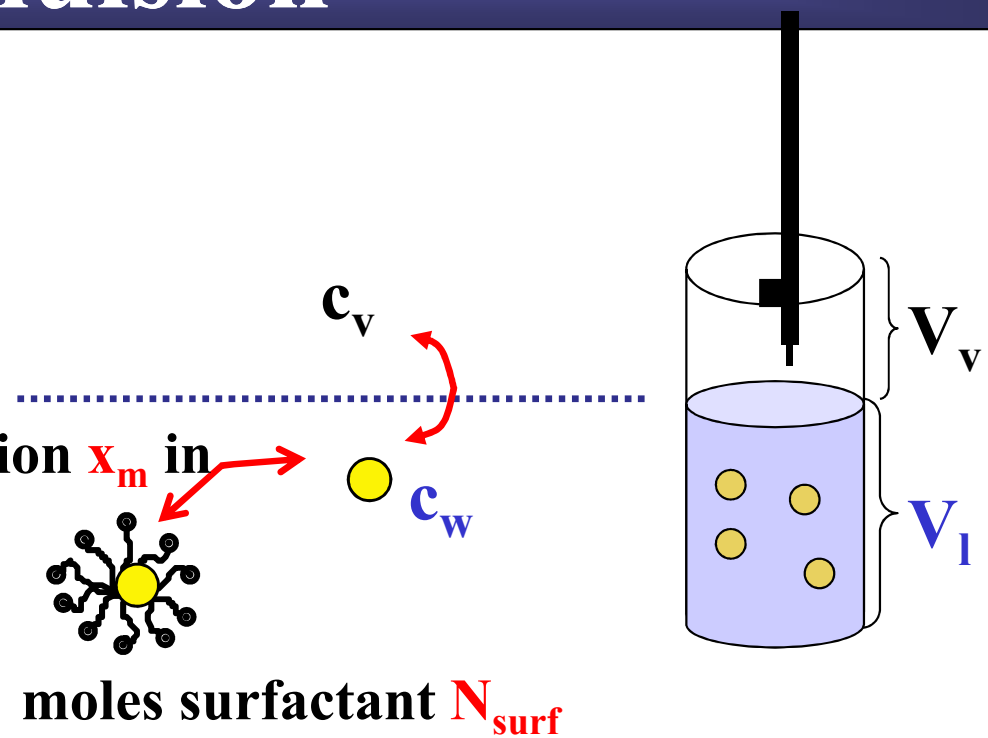
- **Limonene mole balance:**

$$N_{total} = x_m N_{surf} + c_w V_l + c_v V_v$$

- **Define**

$$K_{mic/w} \equiv \frac{x_{mic}}{c_w}$$

$$K_{v/w} \equiv \frac{c_v}{c_w}$$

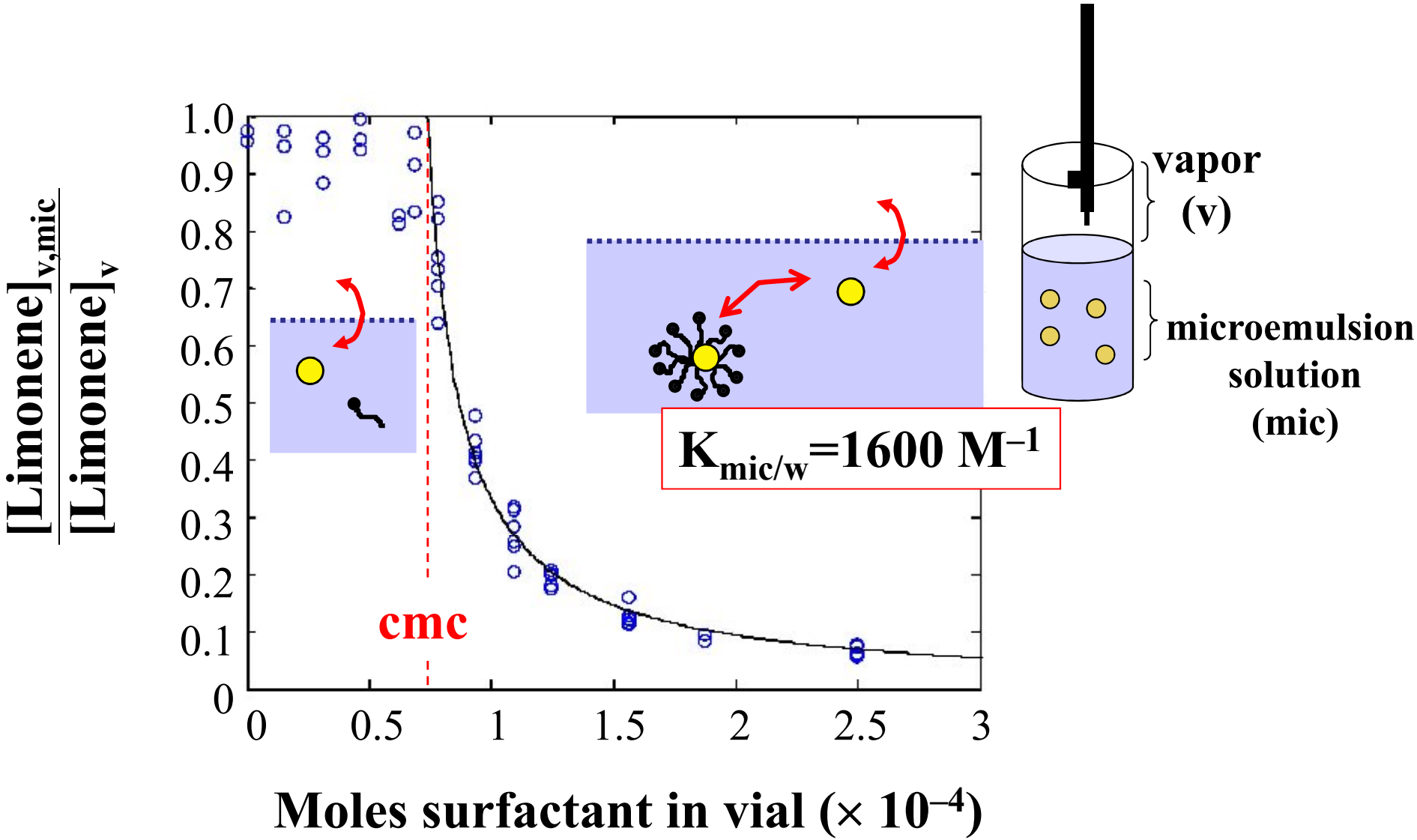



- **Use *headspace* SPME-GC-MS to measure $c_v$**

$$\frac{c_{v,mic}}{c_v} = \frac{V_l + K_{v/w} V_v}{K_{mic/w}(N_{surf} - cmc V_l) + V_l + K_{v/w} V_v}$$

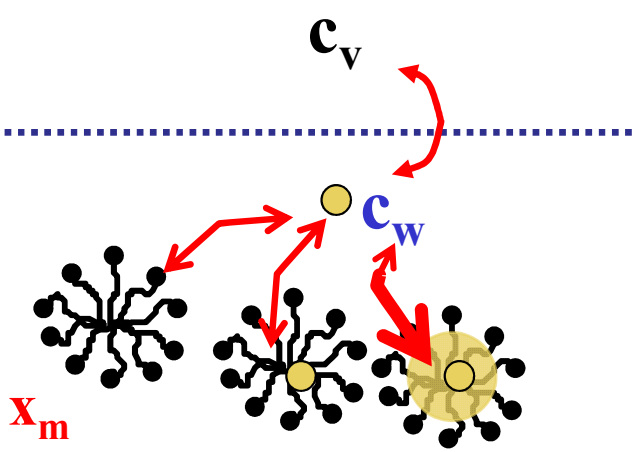
critical micelle concentration

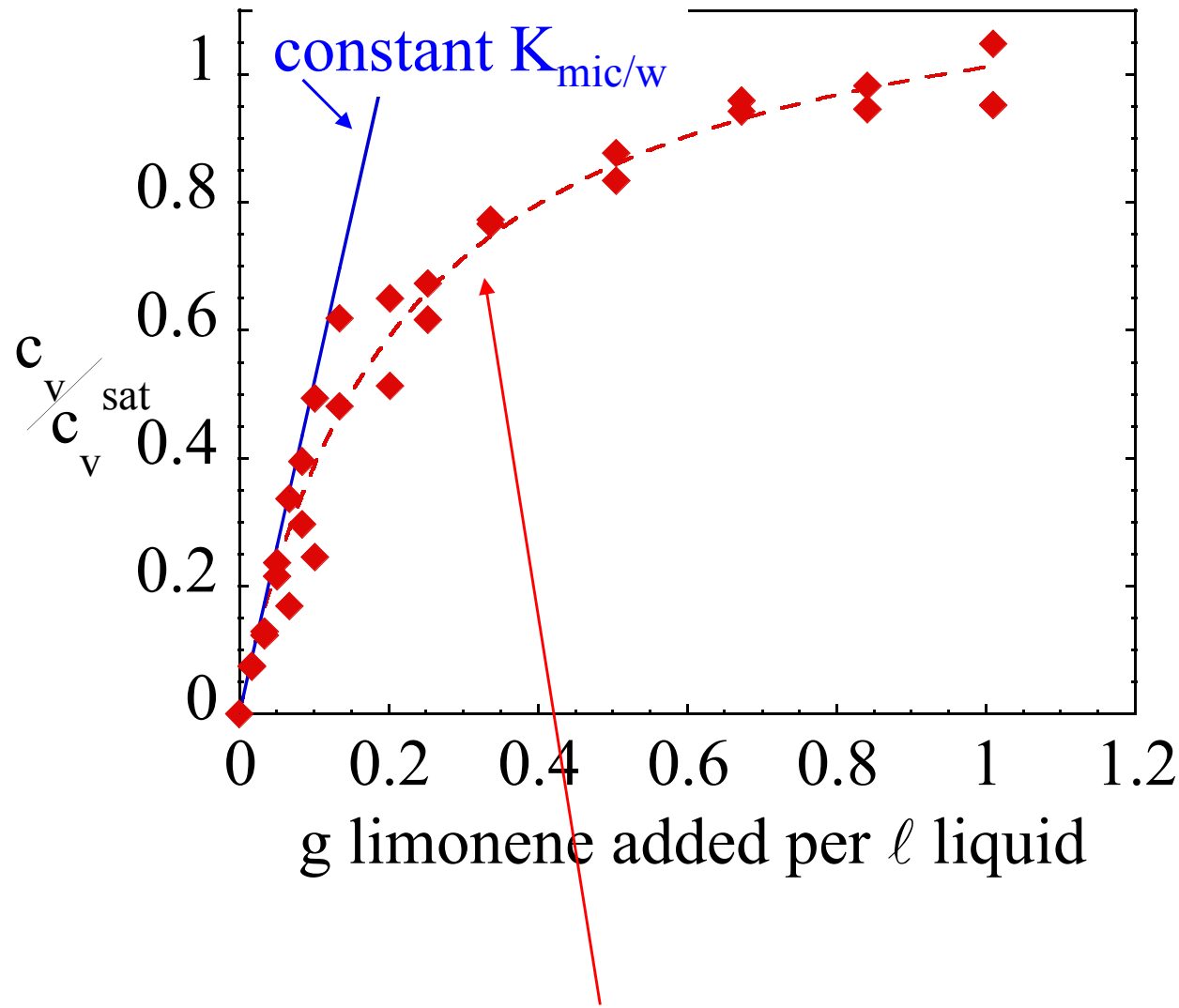
# Effect of Matrix: Microemulsions



# Concentration Effects

$$K_{mic/w} \equiv \frac{x_{mic}}{c_w}$$

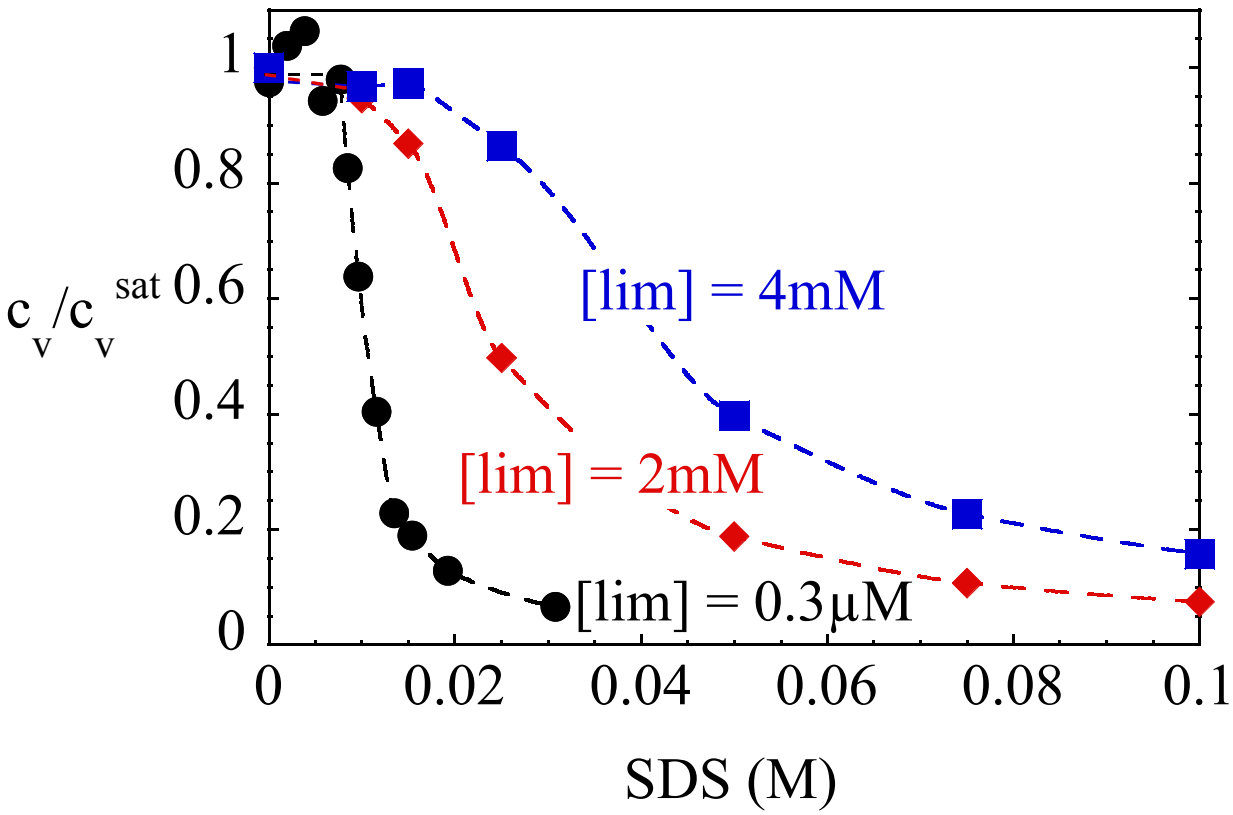



- Partitioning into nanostructure more favored as [limonene] ↑

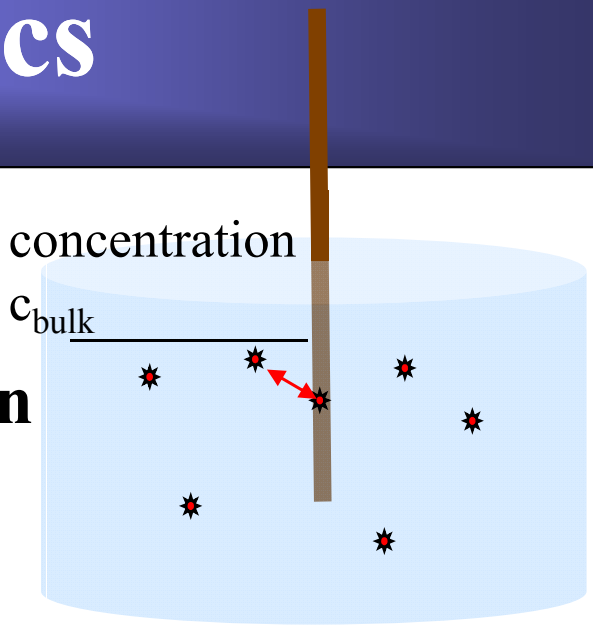
# Vary SDS at Constant Limonene

Probe effects of solute concentration < aqueous solubility limit and >>aqueous solubility limit.

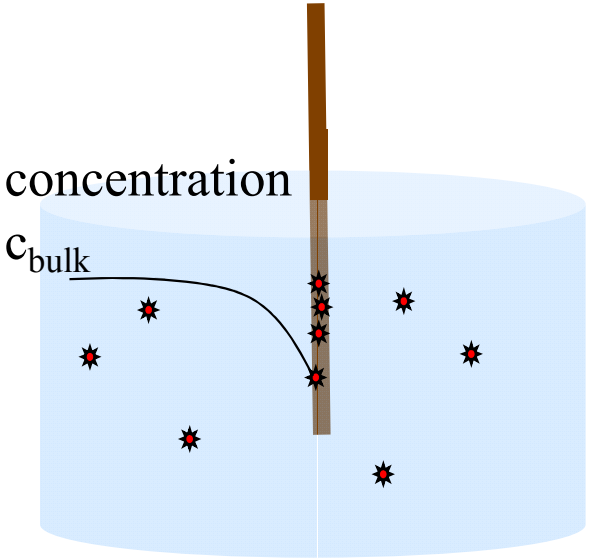


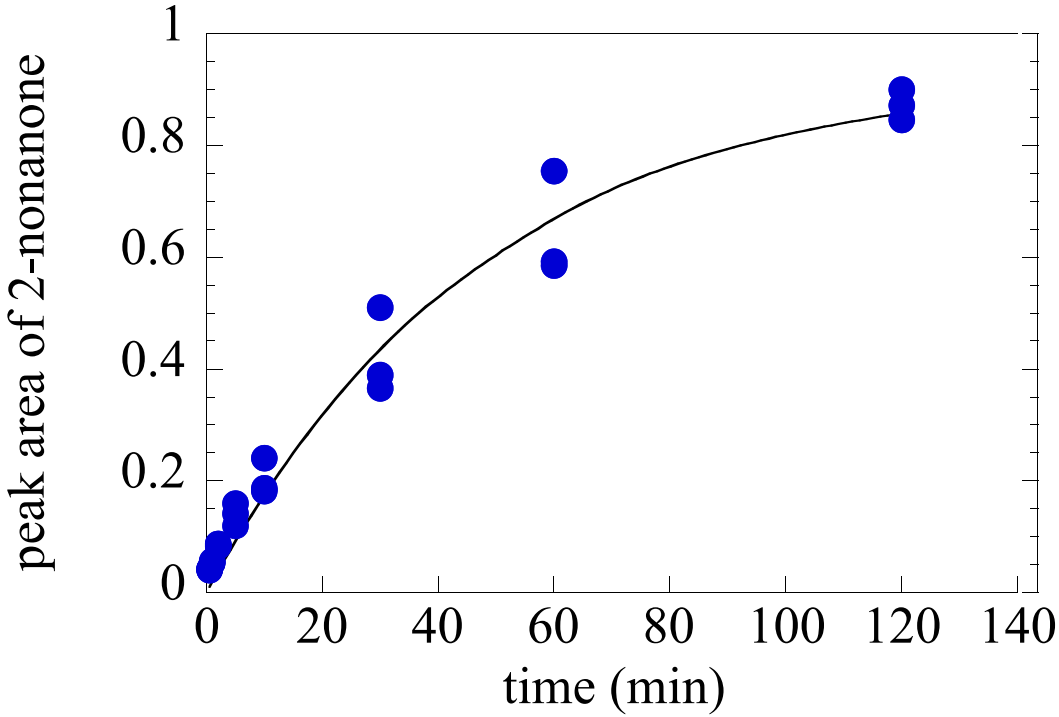
# Measuring Kinetics

- **Short extraction time (1 min)**
  - **sample local, equilibrium concentration**



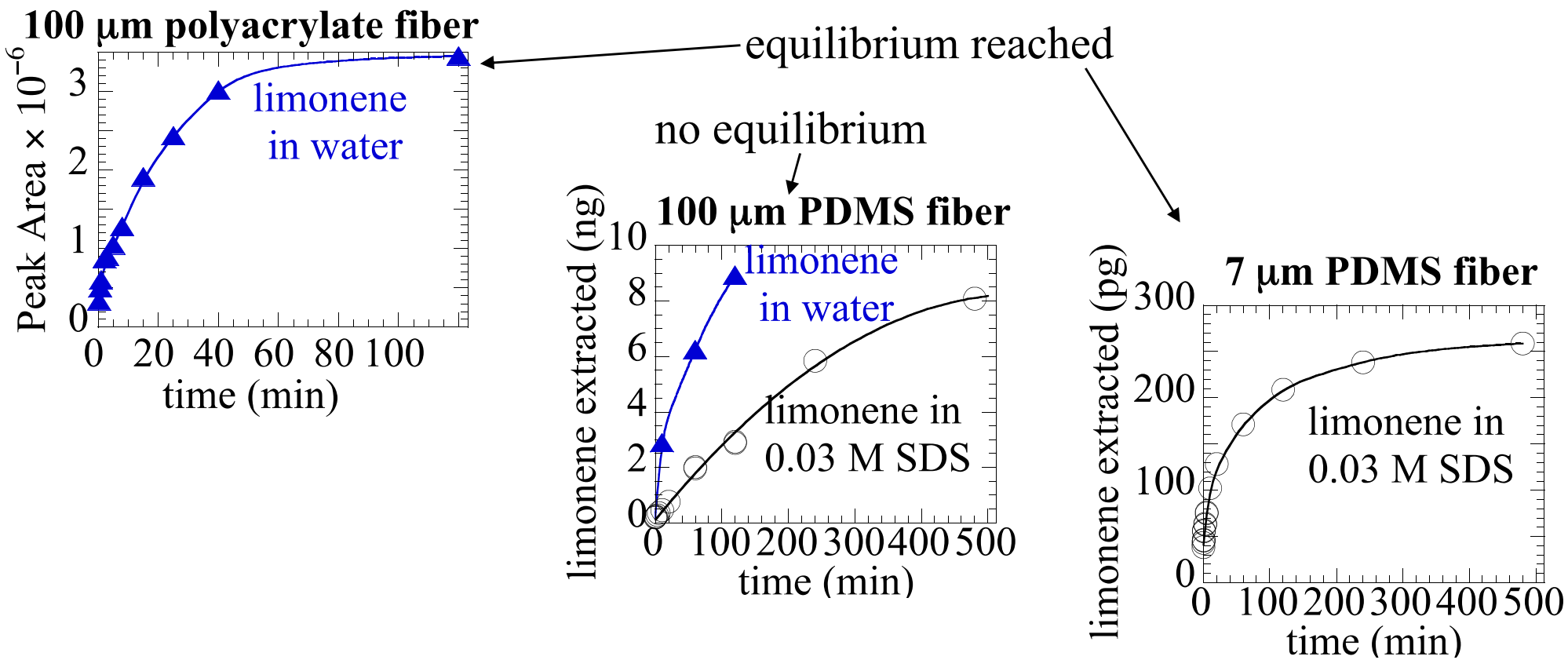

- **Longer extraction times**
  - **shift local equilibrium**
  - **probe kinetics**



# Probing Release from Microemulsions

- Probe different time scales by varying fiber type/thickness



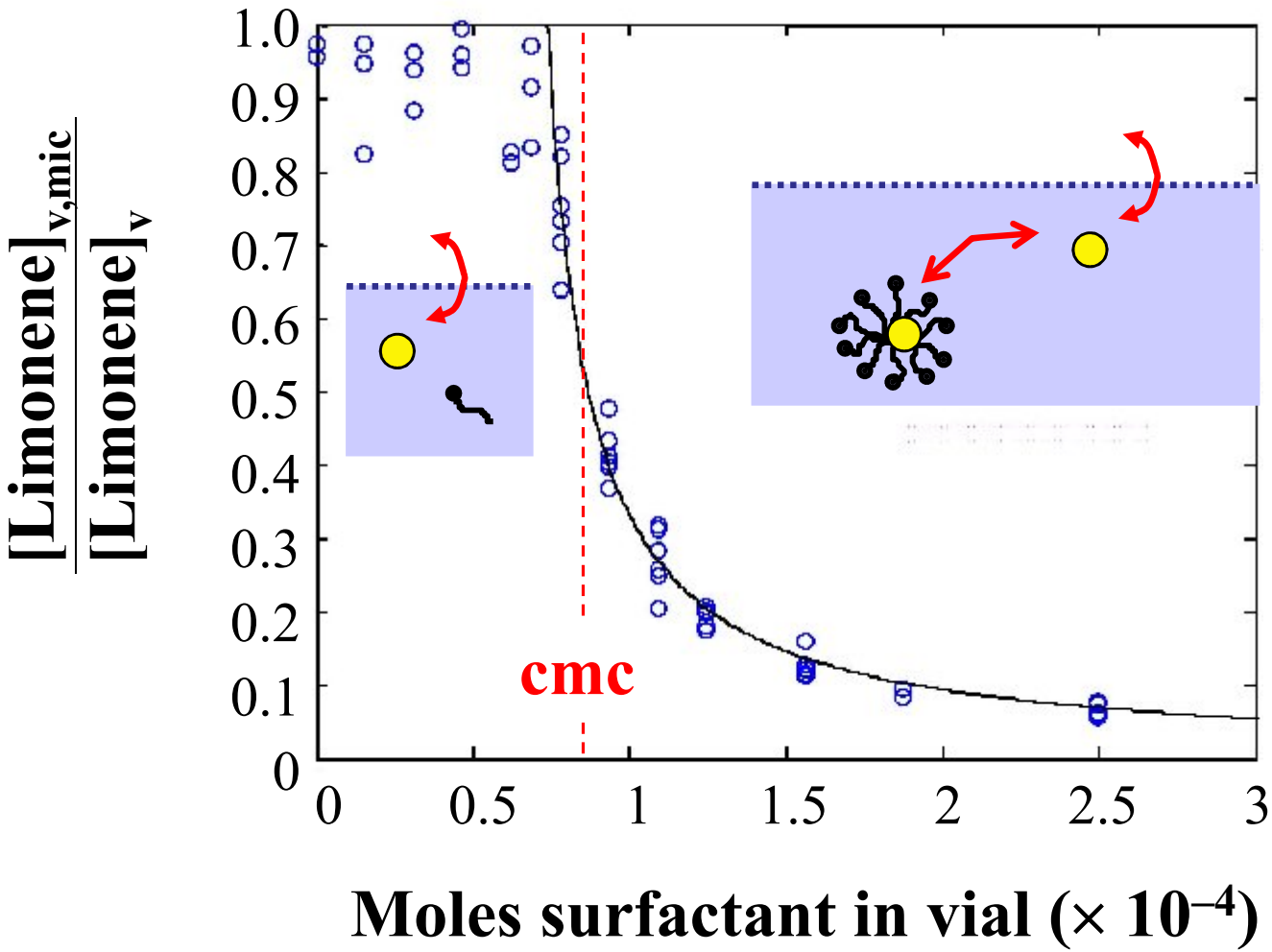

- SDS droplets deplete local aqueous concentrations of limonene
  - release is slower and more persistent

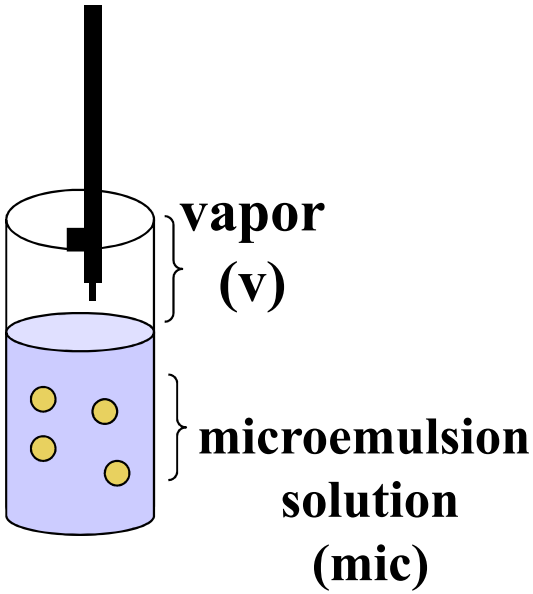
# Summary

- SPME method measures $K_{vl}$ in multiphases
  - Good agreement between HS-SPME vs DI-SPME
  - Over 4 orders of magnitude in $K_{vl}$
  - Independently measure $c_l$ or $c_v$
- Measure partitioning in micellar phases ($K_{ml}$)
  - Surfactant concentration effects
  - Solute concentration effects
- Measure kinetics of release

**Thank you.**

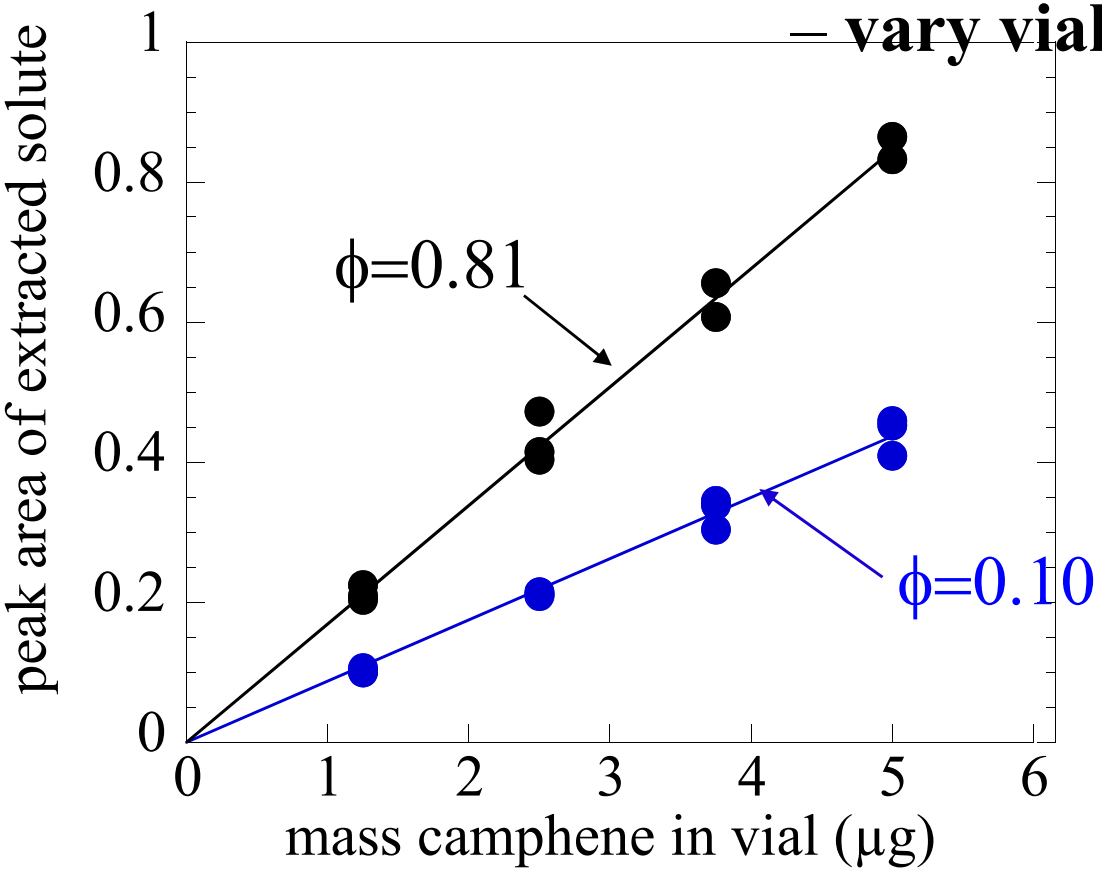
# Microemulsions and Flavor Release

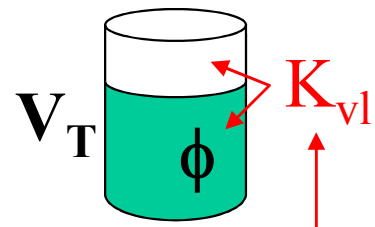


# Partitioning Measurements

- **Extract concentrations in vapor or liquid**
  - **vary volume fraction of liquid $\phi$**
  - **vary vial volume $V_T$**



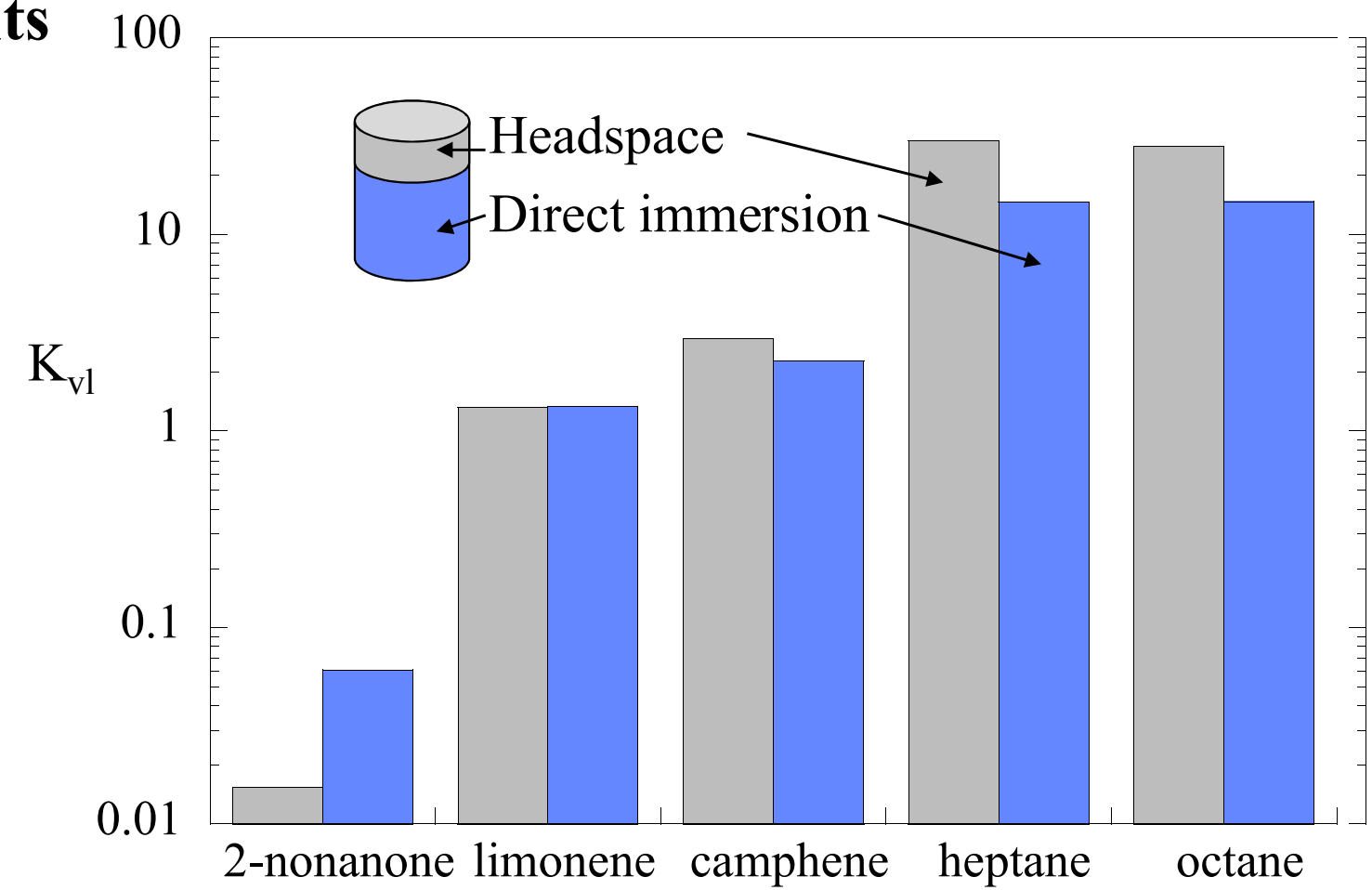

partition coefficient

$$\frac{\text{Slope}_1}{\text{Slope}_2} = \frac{V_T^{(1)}}{V_T^{(2)}} \frac{\phi_2 + K_{vl}(1-\phi_2)}{\phi_1 + K_{vl}(1-\phi_1)}$$

→ **Obtain $K_{vl}$ for flavors of a range of polarities**

# Extraction from Multiphases

- **Consistent results from headspace and liquid measurements**

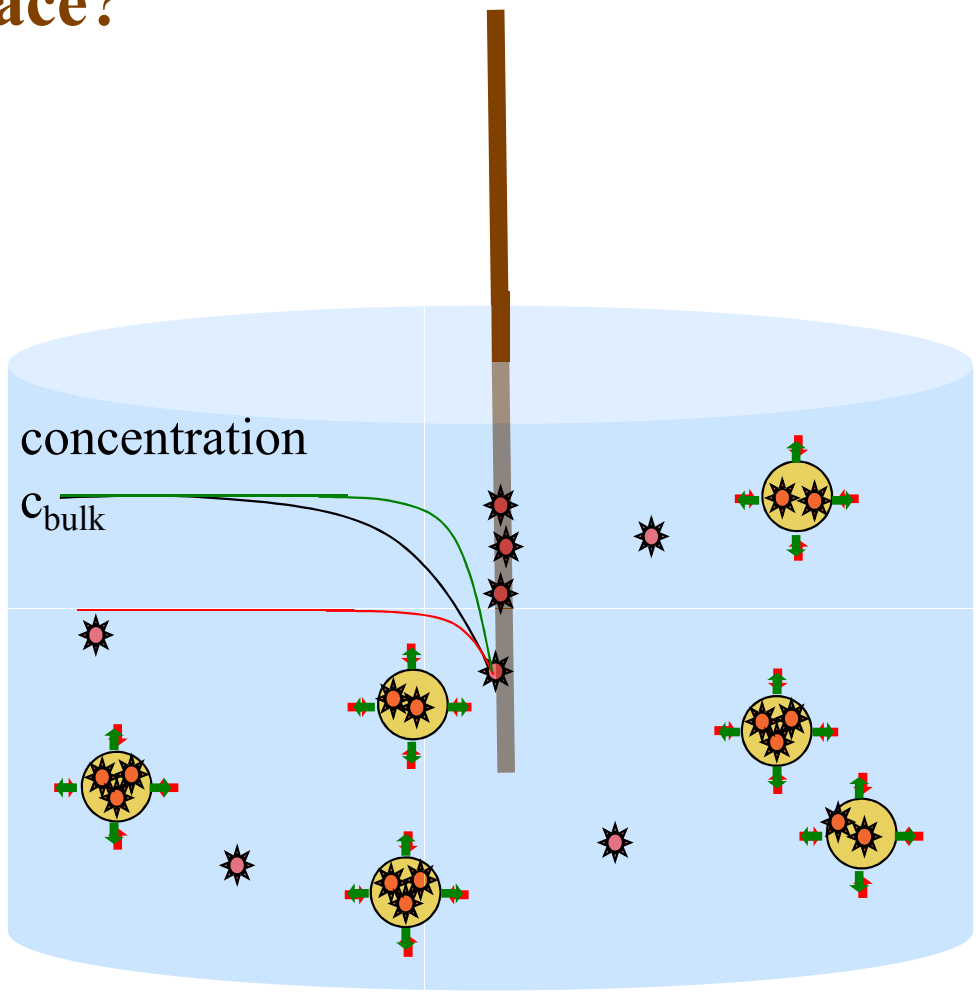


# Effect of Matrix

- **Effect of droplets from emulsion or microemulsion**
  - **resistance at droplet interface?**

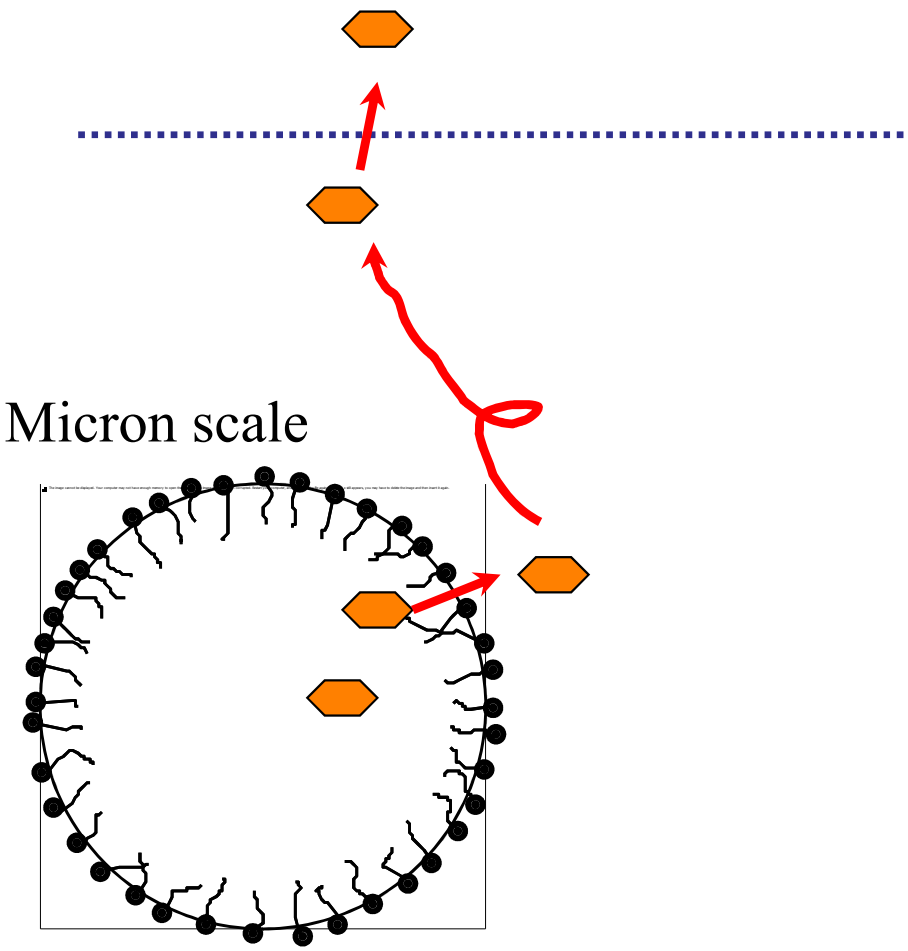
- **If $c_{bulk} > c_{aq}^{sat}$**
  - **droplets maintain aqueous saturation**
  - **accelerate delivery**

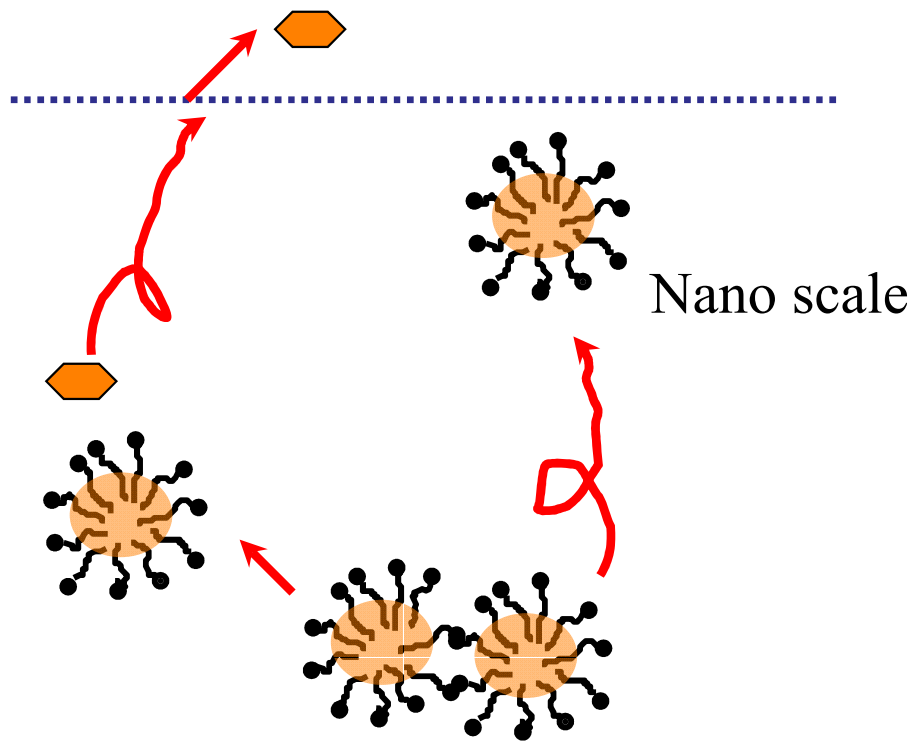
- **If $c_{bulk} \leq c_{aq}^{sat}$**
  - **droplets deplete aqueous phase**
  - **slow delivery**



# Release Mechanisms

- **Emulsions**
- **Microemulsions**




<u>Objective</u>: Develop tools to probe release kinetics
—probe different regions/mechanisms

RUTGERS

THE STATE UNIVERSITY OF NEW JERSEY

# Food Nanotechnology: Understanding the Parameters of Consumer Acceptance


Project Director:

William K. Hallman, PhD.

Director, Food Policy Institute

Chair, Department of Human Ecology

# Co-Investigators

- Dr. Richard D. Ludescher
  - Dean, Cook Campus; Professor, Department of Food Science, Rutgers University
- Dr. Beverly J. Tepper
  - Professor, Department of Food Science, Rutgers University
- Dr. Dietram A. Scheufele
  - Professor of Life Sciences Communication and Journalism & Mass Communication, University of Wisconsin
- Dr. Cara L. Cuite
  - Program Manager, Food Biosecurity Research Program, Food Policy Institute, Rutgers University
- Dr. Mary L. Nucci
  - Program Manager, Food, Media, and Society Research Program, Food Policy Institute, Rutgers University

# Motivation for Study

- Success of food and agricultural nanotechnology depends on both:
  - The ability to manipulate matter at the nanoscale
  - The ability to successfully market the resulting products

- This project is an interdisciplinary effort to facilitate a more thoughtful approach to development of food nanotechnology, based on consumers' preferences and concerns

# Significance

- Most studies of consumer perceptions of nanotechnology have focused on the acceptability of the *technology* and have done so in the abstract
- Few studies of nanotechnology have examined applications of the technology to food products
- This series of studies examines consumer perceptions of food nanotechnology by examining the important parameters that influence the acceptability of products

# Expected Outcomes of Overall Study

Understanding of acceptable characteristics and uses of nanomaterials in food

- Establish the baseline parameters of acceptability of nanotechnologies in food.
- Understand *who* may be most open to nanotech foods
- Understand factors that influence acceptance

# Overall Project Research Design

Coordinated set of studies examining consumer reactions to realistic nanotech food products

- Qualitative interviews
  - Completed, manuscripts under review
- National survey
  - Completed, manuscripts to be submitted in January
- Food sensory experiment
  - Completed, analyses in progress

# Qualitative Interviews

# Qualitative Interviews - Methods

- Individual interviews with 31 adults in 2009
  - Each lasted between 1.5 and 2 hrs.
- Elucidated thoughts about nanotechnology in general, and food applications in particular
- Read and evaluated NNI Nanotech Booklet
- Participants tasted conventional food products identified as containing nanotechnology
- Also measured expressed willingness to try or buy nine hypothetical nanotechnology food products with various benefits

# Results: Knowledge was Low

- Consistent with previous studies (52%), more than half noted they did not know much about nanotechnology
  - However, 58% were able to link nanotechnology to small size
- No participant able to describe key attributes of nanotechnology
  - Generate and make use of novel properties of material at molecular level
- Only twenty-two (22%) percent could think of any connections between nanotechnology and food even after reading the NNI brochure

# Qualitative Interviews - Results

- Nearly all were willing to *try* the products, few said they would *buy* them.
- Less than 10% expressed negative perceptions of food-related nanotechnology

# Qualitative Interviews - Significance

- Specific application, benefits, price, hedonic and sensory qualities of food products had greater influence on participant responses than understanding, approval, or disapproval of the technology itself
  - However, future purchasing decisions are likely to be strongly dependent on perceived uncertainty about the long-term effects of eating nanotechnology-based food products
- Confirms previous research that acceptance of food nanotechnology is unlikely to be driven by product benefits alone

# National Survey

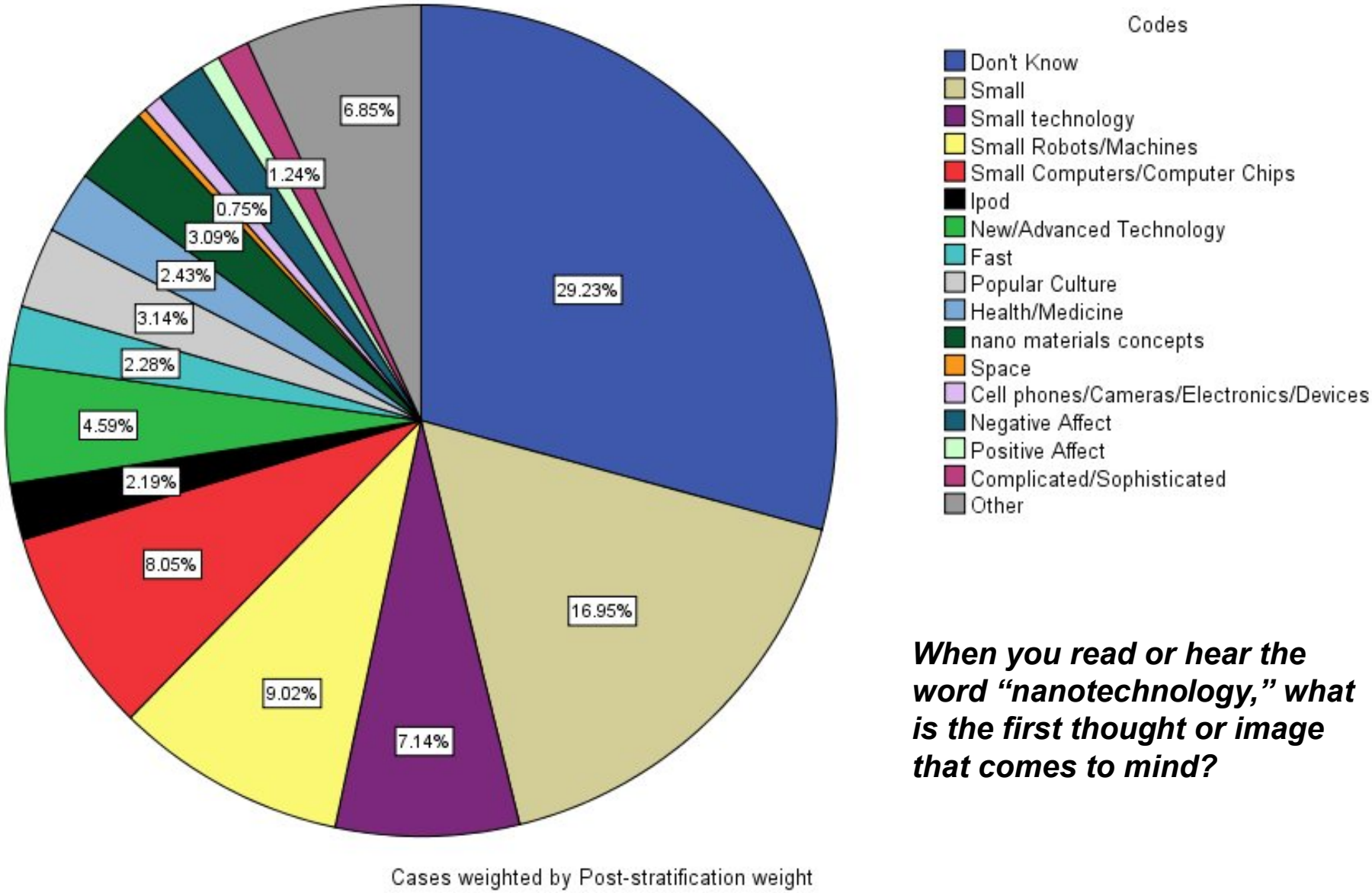
# National Internet Survey

- Survey conducted between April 9, 2010 and April 27, 2010.
- 1836 Knowledge Networks panelists aged 18 and over were invited to participate.
- 1210 panelists completed the survey online.
  - 66% completion rate

# Overview of National Survey

- Questions about respondent starting points.
- Nanotechnology was defined in three different ways; intentions to purchase and consume nano-based food products were measured.
- A long series of T/F questions about nanotechnology followed, and then a second experiment, fully crossed with the first.
- An apple was described that had nanotechnology incorporated into it in three different ways.
- Finally, respondents were asked to provide assessments of a range of potential food nanotechnology products.

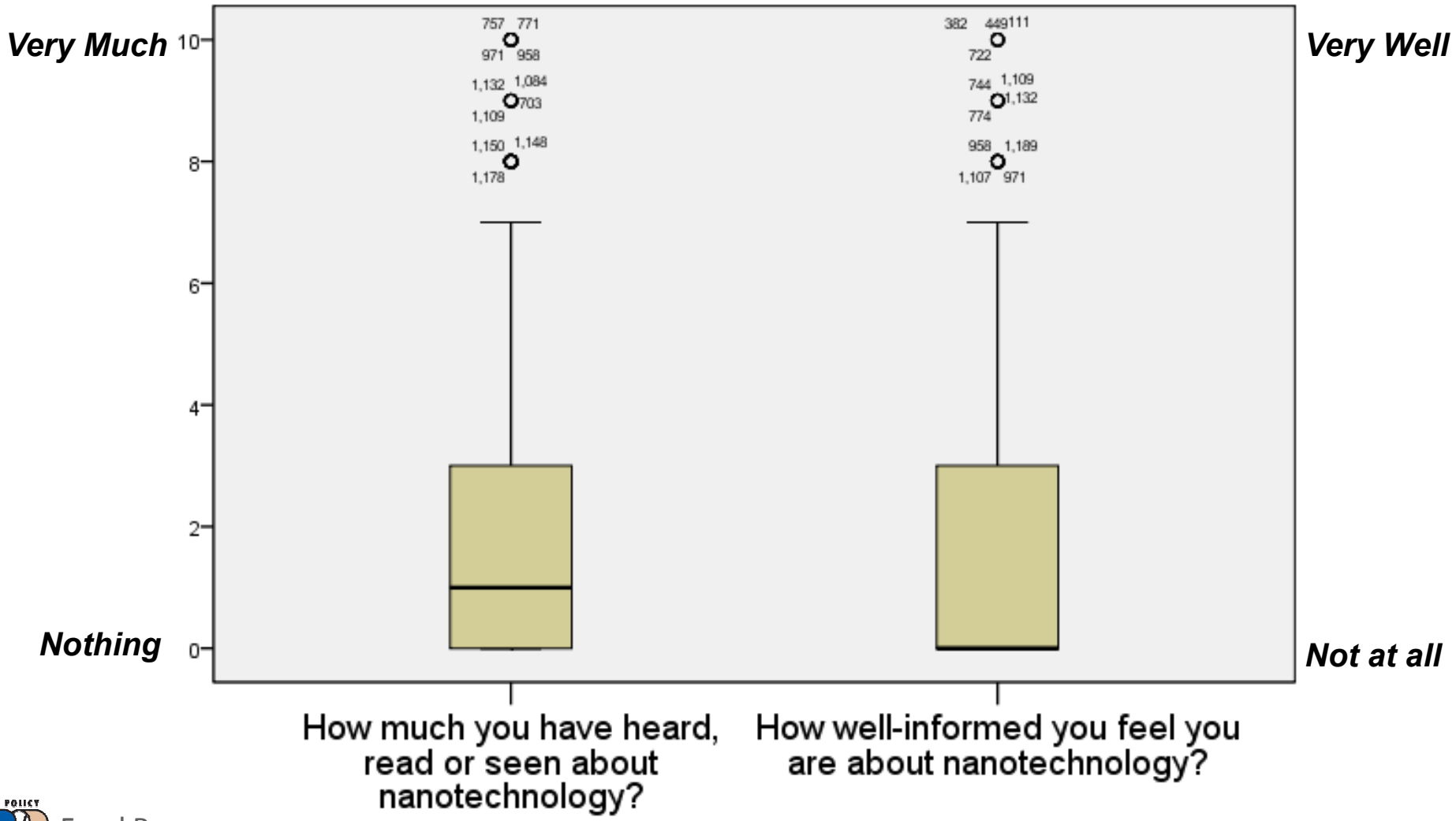
# What Comes to Mind? - Nanotechnology




***When you read or hear the word "nanotechnology," what is the first thought or image that comes to mind?***

# People Know Little about Nanotechnology

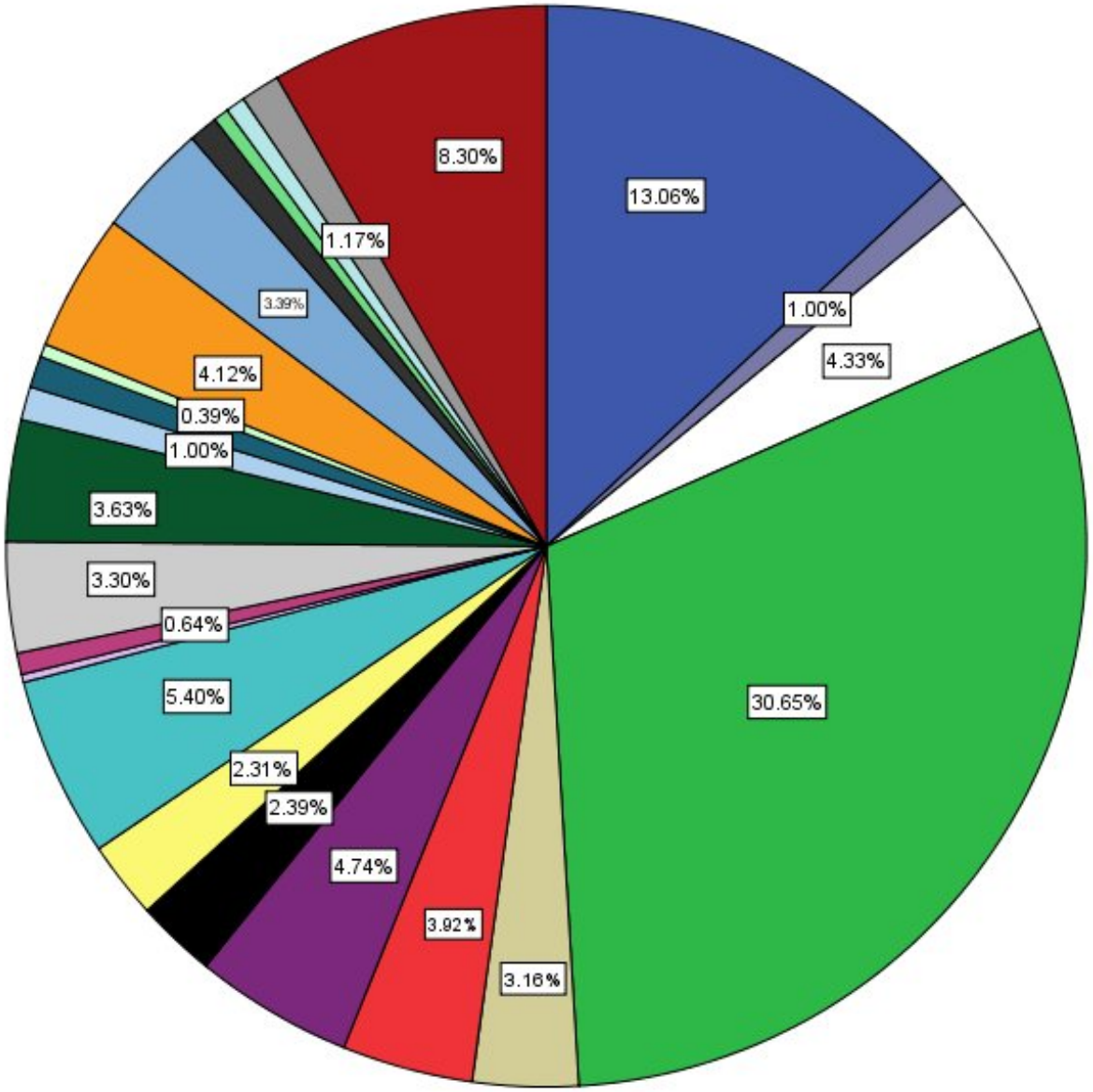
***"Nanotechnology allows scientists to manipulate materials at the level of tiny molecules."***



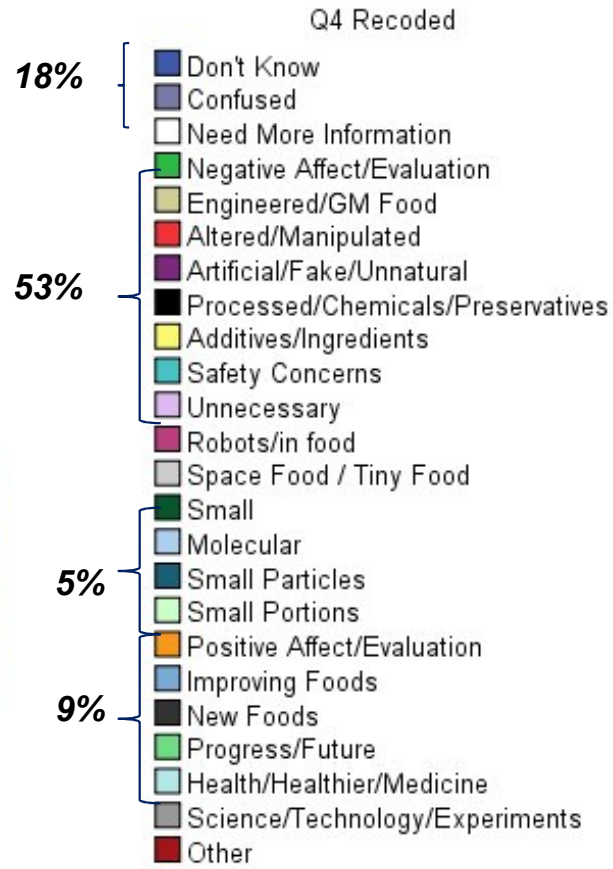

Food Policy Institute

Cases weighted by Post-stratification weight

# Food Nanotechnology?




Cases weighted by Post-stratification weight




***When you read or hear the word "food nanotechnology," what is the first thought or image that comes to mind?***

# Experiment 1: Description of Nanotechnology

- Between subjects factor
- Three levels of description of nanotech:
  - Nanotech is small
  - Nanotech is different
  - Nanotech is unpredictable

# Small Description

**Nanotechnology materials are extremely small.**

Nanotechnology allows scientists to make and use extremely small materials. These particles are measured in nanometers. One nanometer is one-billionth of a meter. It may be difficult to fully understand just how small a nanometer is. For example, a single human hair is around 80,000 nanometers in width, a single sheet of paper is 100,000 nanometers thick, and the head of a pin is about 1 million nanometers across. Because the materials are so small, it is possible to create mixtures of oil and water that never separate, to add particles that never sink to liquids, and to add ingredients that won't change the taste of foods. The fact that these materials are so small makes it possible to create many new kinds of food products.

# Different Description

**Nanotechnology materials behave differently than their larger counterparts.**

Nanotechnology allows scientists to make and use extremely small materials. What is important about these small materials is that they act very differently on the nano scale than they do on a larger scale. As a result, even common materials used in foods can have very different physical or biological characteristics when they are used at the nanoscale. Materials made with nanotechnology can also have different chemical reactions and biological properties compared with the same materials when they are not so small. For example, through the use of nanotechnology, it is possible to create mixtures of oil and water that never separate, to add particles that never sink to liquids, and to add ingredients that won't change the taste of foods. The different properties of these nano scale materials make it possible to create many new kinds of food products.

# Unpredictable Description

**Effects of nanotechnology materials are unpredictable.**

Nanotechnology allows scientists to make and use extremely small materials. What is important about these small materials is that they act very differently on the nano scale than they do on a larger scale. Even common materials used in foods can have very different physical, chemical, and biological properties when they are used at the nano scale. For example, through the use of nanotechnology, it is possible to create mixtures of oil and water that never separate, to add particles that never sink to liquids, and to add ingredients that won't change the taste of foods. The different properties of these nano scale materials make it possible to create many new kinds of food products. However, these different properties and their very small size also make it much more difficult for scientists to accurately predict how nano materials might affect people and the environment.

# Behavioral Intention Response Scale

- A series of manipulation checks indicated that the interventions worked as intended.
- For a dependent measure, we created a Behavioral Intention Scale combining four items:
  - I would purchase foods labeled as containing 'nanotechnology' in the grocery store.
  - I would eat nanotech foods.
  - I would serve nanotech foods to my family and friends.
  - I would recommend nanotech food to a friend.
- 0 = Strongly Disagree and 10 = Strongly Agree (11pt. scale)
- Lower scores indicates less acceptance
- Cronbach's alpha = .96

# ANOVA for Nano Description

- $F(2, 1190) = 3.99, p>.05$

| | Description | | |
|---|---|---|---|
| | Small | Different | Unpredictable |
| Mean | $2.53_{a}$ | $2.25_{a,b}$ | $2.06_{b}$ |

***Means that don't share the same subscript are significantly different at p<.005; Lower score indicates less acceptance. Scale from 0 to 10.***

# Experiment: Where in the food is the nanotechnology?

**Between subjects factor; fully crossed with conditions from earlier experiment; subjects saw ONE of the following descriptions**

**Grown into**

- A recently developed type of nanotechnology helps to preserve the freshness of apples. Nanotech particles are added to the soil. The nanotech particles are taken up by the roots of the tree and become part of the apple.

**Applied outside**

- A recently developed type of nanotechnology helps to preserve the freshness of apples. Nanotech particles are applied to the outside of the apple after it is picked from the tree. The nanotech particles can be washed off or taken off if the skin of the apple is removed.

**In packaging**

- A recently developed type of nanotechnology helps to preserve the freshness of apples. Nanotech particles are added to the plastic bags manufactured to hold apples. The nanotech particles remain in the plastic bag and do not transfer to the apples.

# Response scale included 12 items:

- This type of apple would be fresh.
- This type of apple would be nutritious.
- This type of apple would be safe for the environment.
- This type of apple would be safe for human health.
- This type of apple would taste very good.
- This type of apple should be included in the school lunch program.
- If it met the other existing standards, this type of apple should be considered "organic."
- This type of apple should be sold in natural food stores.
- I would purchase this type of apple in the grocery store.
- I would eat this type of apple.
- I would serve this type of apple to my family and friends.
- I would recommend this type of apple to a friend.

0 = Strongly Disagree and 10 = Strongly Agree;(11pt scale); lower scores indicates less acceptance; Used the mean of the eleven items

Cronbach's alpha = .97

# ANOVA for effects of apple nanotechnology location on behavioral intentions

F (2, 1092) =6.94, p>001.

| | Location of nano in apple | | |
|---|---|---|---|
| | Grown into | Applied outside | In packaging |
| Mean | $3.96_a$ | $3.66_a$ | $4.32_b$ |

***Means that don't share the same subscript are significantly different at p≤.05; Lower score indicates less acceptance. Scale from 0 to 10.***

# Conclusions from Experiment

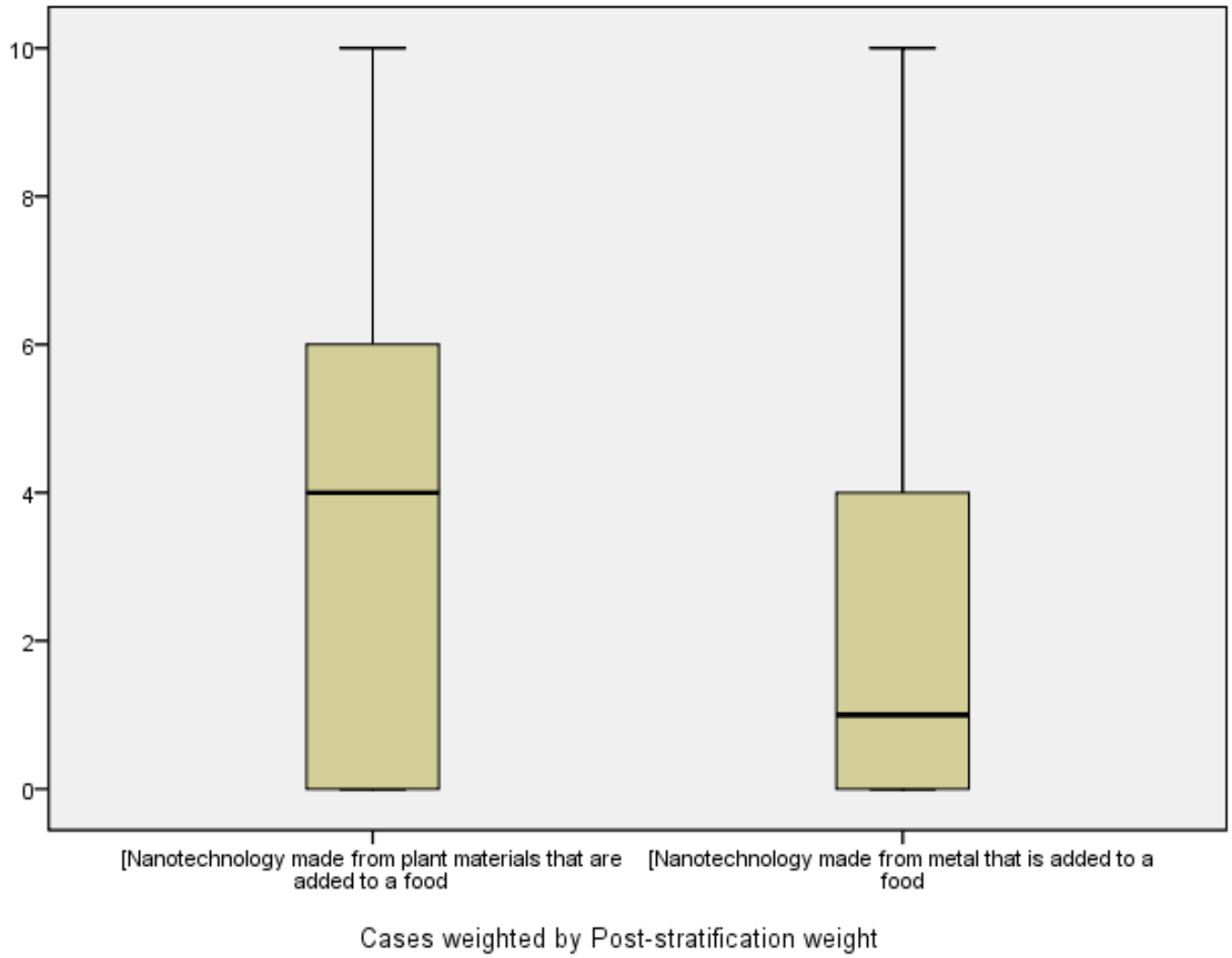
- The key feature affecting acceptance is whether the nanotechnology is in any way incorporated into the food.

# Evaluations of other nanofood products

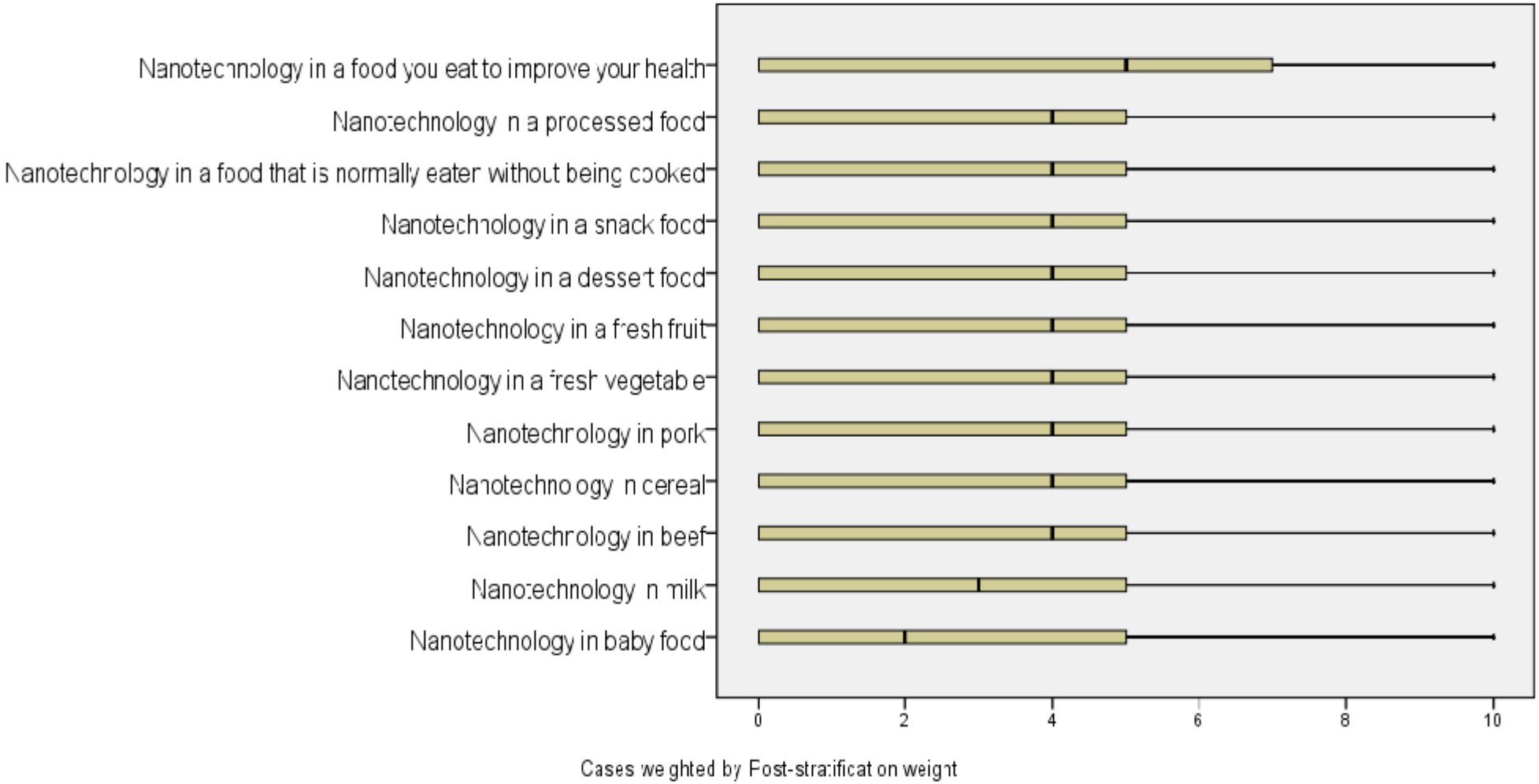
Nanotechnology can be used with food in a number of different ways. On the following screens, please rate how much you approve of the following types of nanotechnology food products.

- 0=do not approve at all
- 10=totally approve
- No DK option provided

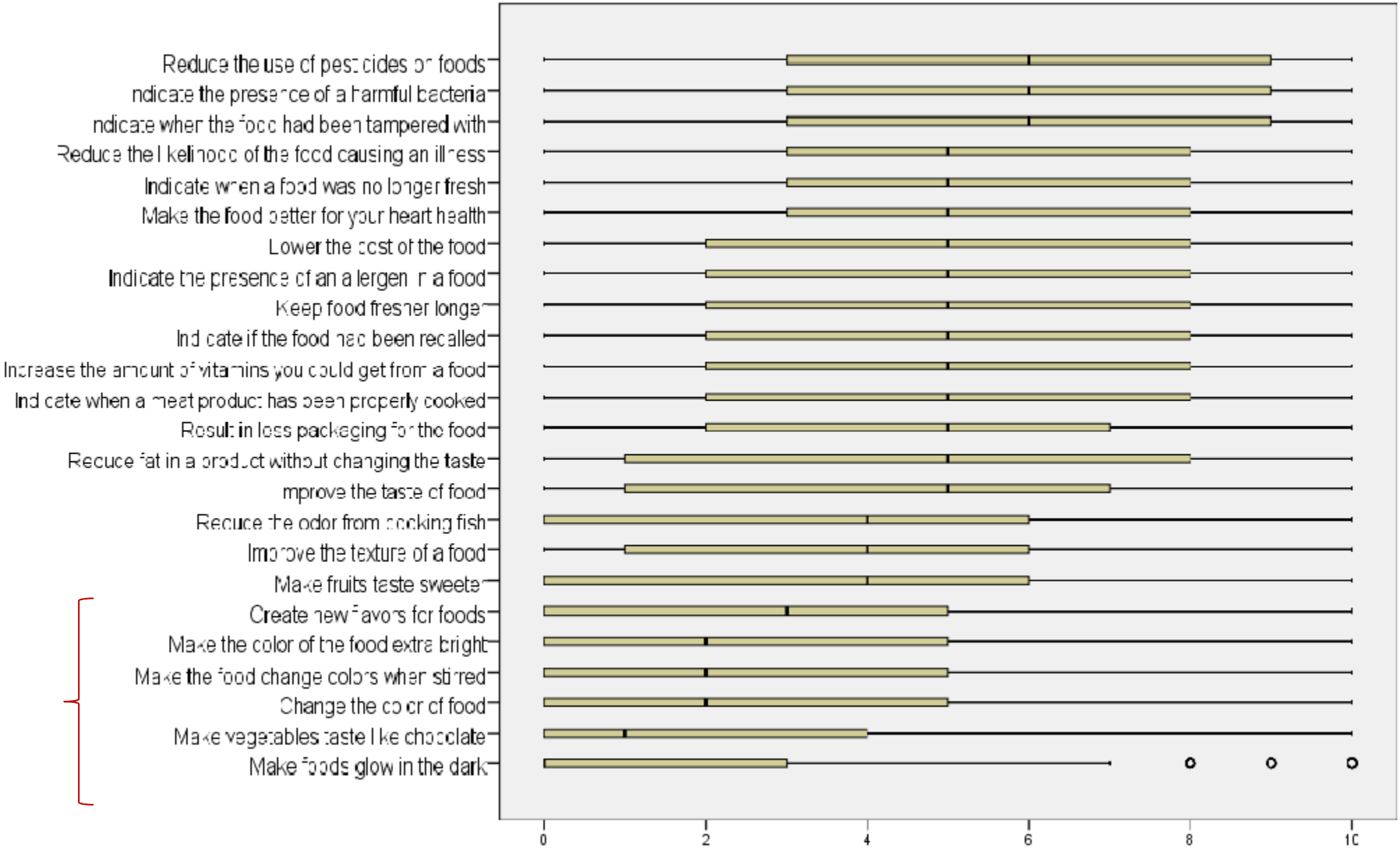
# Preference for Nanotech Materials



# Preference for Nanotechnology in Different Types of Foods



# Rating of Benefits



# Public Prefers "Matched" Product/Benefit

| | Mean | |
|---|---|---|
| Peanut Butter with Omega-3 fatty acids to improve heart health | 5.98 | $t$ (1177) = -7.72, p<.000 |
| Hot dogs with Omega-3 fatty acids to improve heart health | 5.53 | |
| | | |
| Yogurt with fiber to improve digestion | 5.69 | $t$ (1172)= 2.24, p<.05 |
| Ice cream with fiber to improve digestion | 5.54 | |
| | | |
| Bottled water with calcium to improve bone health | 5.54 | $t$ (1172)= -5.45, p<.000 |
| Carbonated Soft Drinks with calcium to improve bone health | 5.19 | |

# Conclusions

- Initial results indicate extremely low levels of acceptance of nanotechnology in food.
- How nanotechnology is described can affect acceptance levels.
- "Nano-on" may be the same as "Nano-in".
- Nanotech materials matter.
- Describing benefits of food nanotechnology increases acceptance somewhat.
- Some benefits seem more desirable than others.
- Benefits need to match the foods they are in.

# Food Sensory Experiment

- Objective:
  - Understand consumer attitudes toward food nanotechonlogy through their reactions to the experience of tasting "nanotech" foods.
- Experimental Design:
  - Consumer acceptance test for two types of foods with or without specific nanotech benefits.
    - In reality, none of the foods were produced with nanoscale materials
- Participants:
  - 161 Total

    44 men

    117 women

# Design

| Type of Food | Control Sample | Health Benefit | Freshness Benefit | Delivery Mechanism |
|---|---|---|---|---|
| Cherry tomatoes | No nanotechnology | Anti-microbial | Stays fresh longer | In the package |
| Chocolate ice cream | No nanotechnology | Provides probiotics | Preserves freshness and prevents icing | In the food |

# Measures

- Liking ratings for key sensory attributes and overall liking
- Surveys for knowledge and attitude towards nanotechnology; general food attitudes
  - Knowledge about nanotechnology
  - Food technology neophobia scale
  - Food choice questionnaire
  - Food nanotechnology attitudes scale

# Results

- Analyses underway
- Willingness to purchase:
  - Tomatoes with nanotech benefits: 74.3 – 82.0%
  - Ice cream with nanotech benefits: 79.5 – 85.7%

## For more information contact:

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The opinions expressed are those of the authors and do not necessarily reflect official positions or policies of the USDA, the New Jersey Agricultural Experiment Station, or of the Food Policy Institute, Rutgers, the State University of New Jersey.

afmnet

ADVANCED FOODS & MATERIALS NETWORK

*Inspiration from the ground up*

# Nanostructural engineering of lipid networks


## Gianfranco Mazzanti

**Dalhousie University, Halifax, Canada**

# Industrial processing of bulk fats: made to last until properly destroyed

- Products
  - Butter (milk fat), margarines, baking shortenings, fractions (olein, stearin, etc.), Chocolate (cocoa butter)
  - **Remove bad Trans-fats** (**Afmnet  Gen. Mills project**)
  - Add good Omega-3
- Processing: Materials **spontaneously form crystalline nanoplatelets**
  - Progressive crystallization:
    - Melt ➔ Suspension ➔ paste ➔ solid matrix
  - For instance the two-step Votator
    - Intense mixing during fast cooling & "Working the fat"
  - Fractionation of components

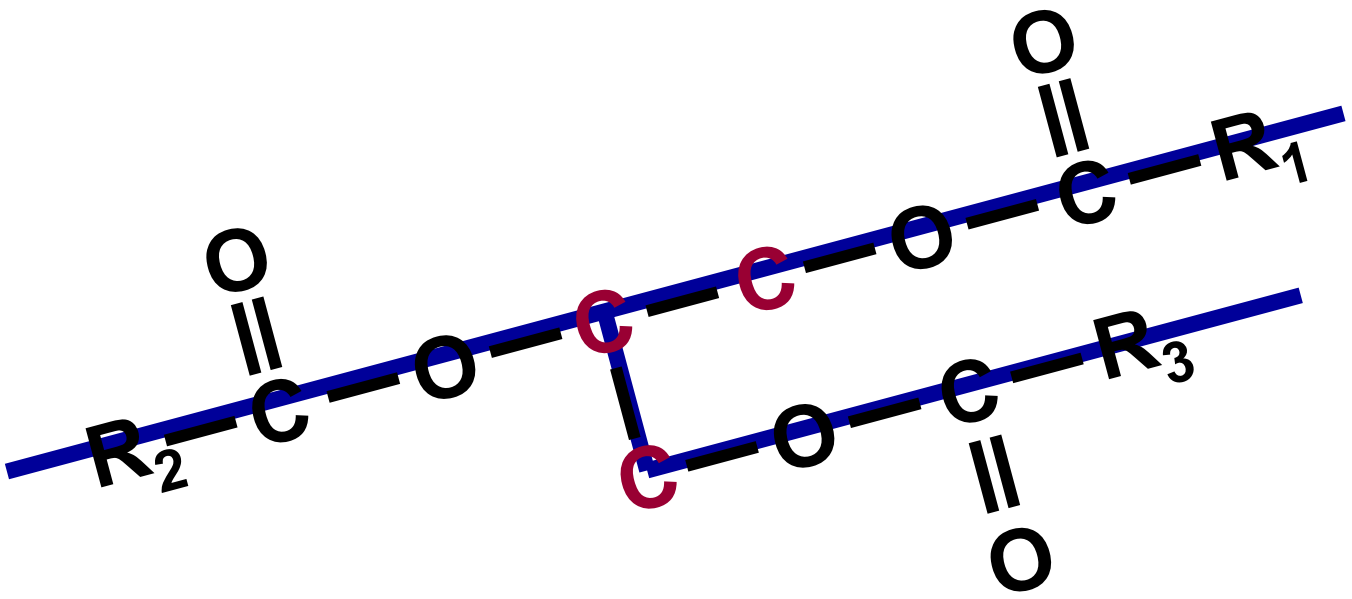
# Engineering design requires

- Understanding nanostructures and their formation processes
- Building predictive models from the knowledge acquired, combining the different pieces of knowledge
- Test models at benchtop and plant level
- Feedback to tune models

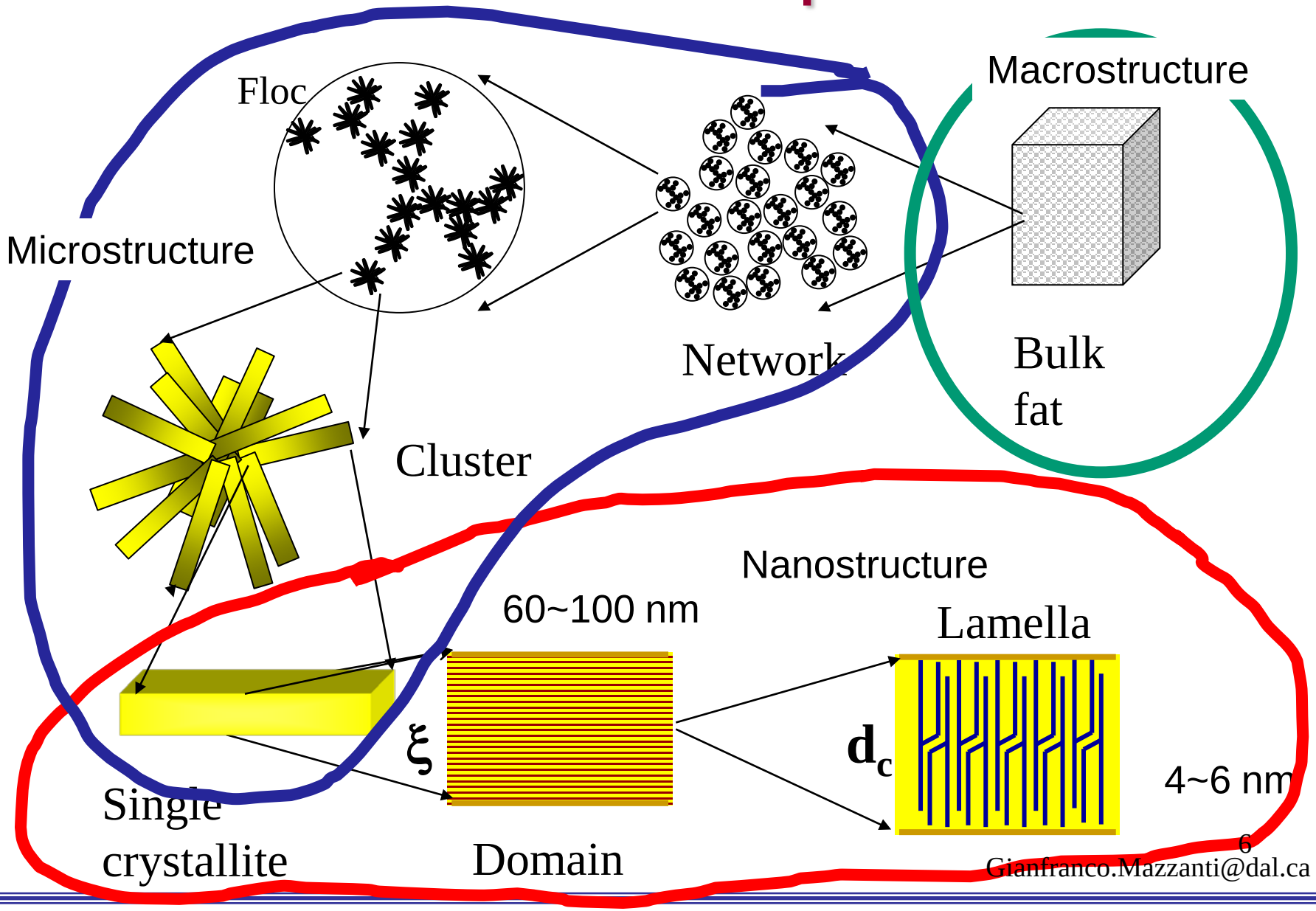
1. X-ray diffraction and electron microscopy

# Understanding nanostructures and their formation processes

# Chemical structure of lipids in fats



# Levels of structure in plastic fats





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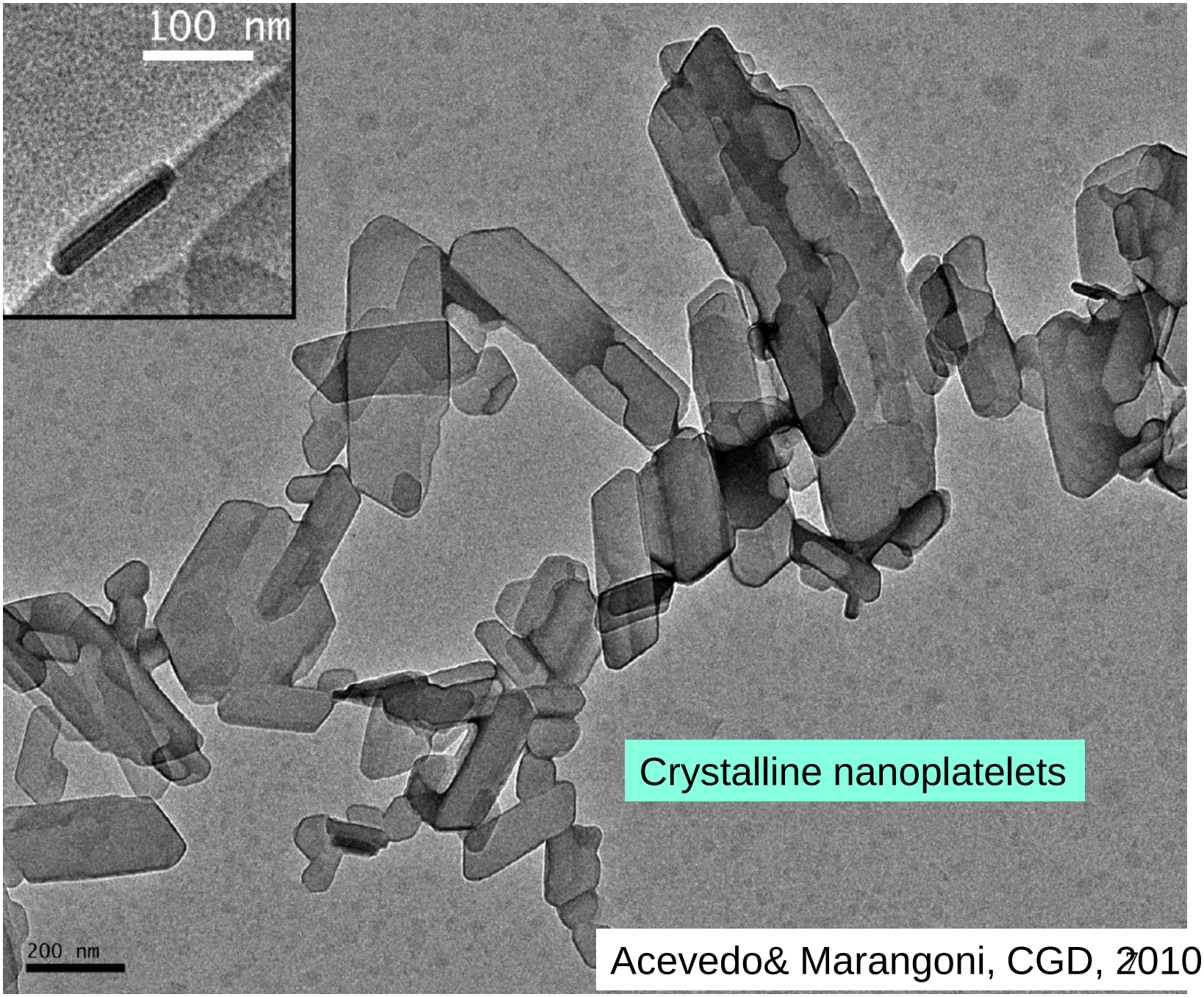
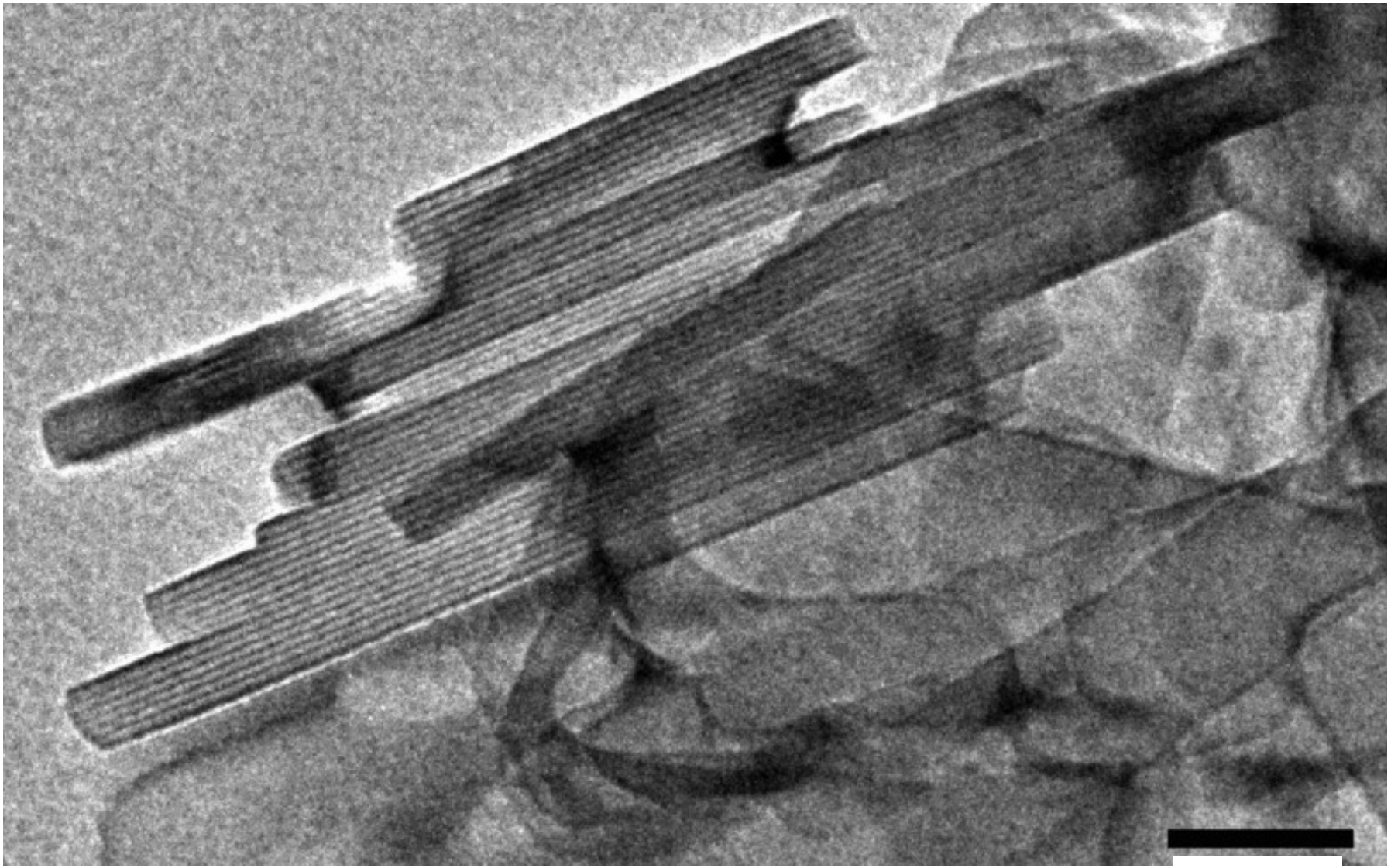



Acevedo& Marangoni, CGD, 2010




Acevedo& Marangoni, CGD, 2010

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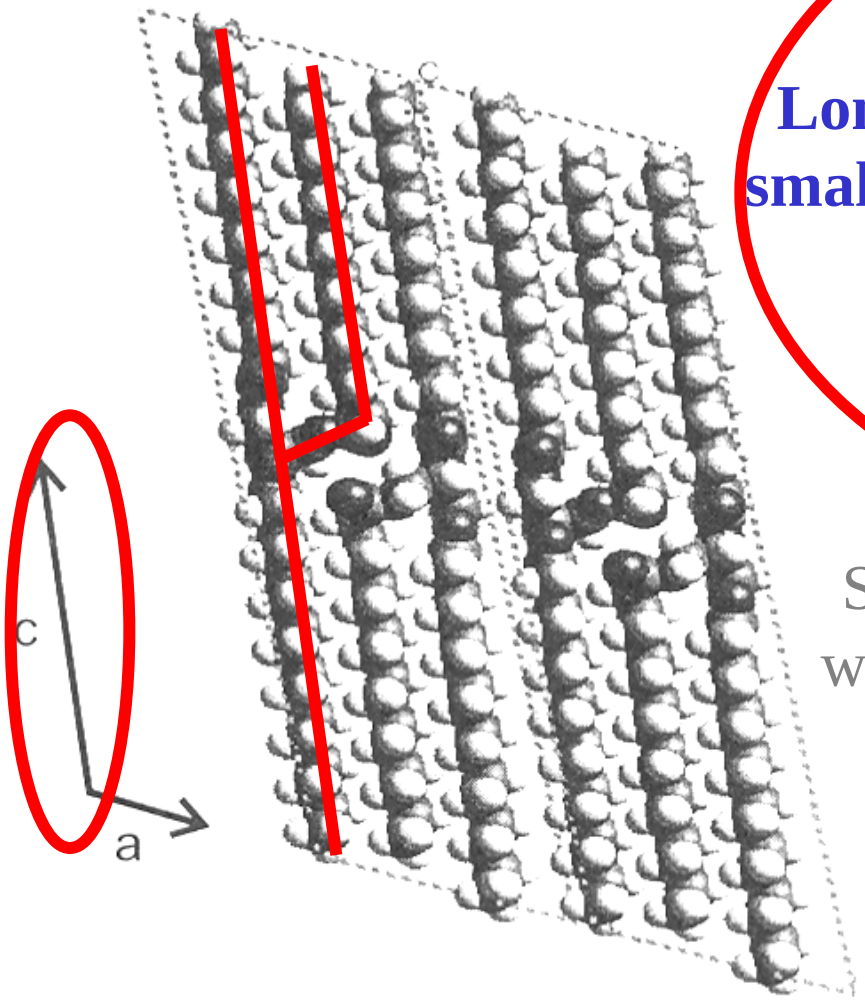
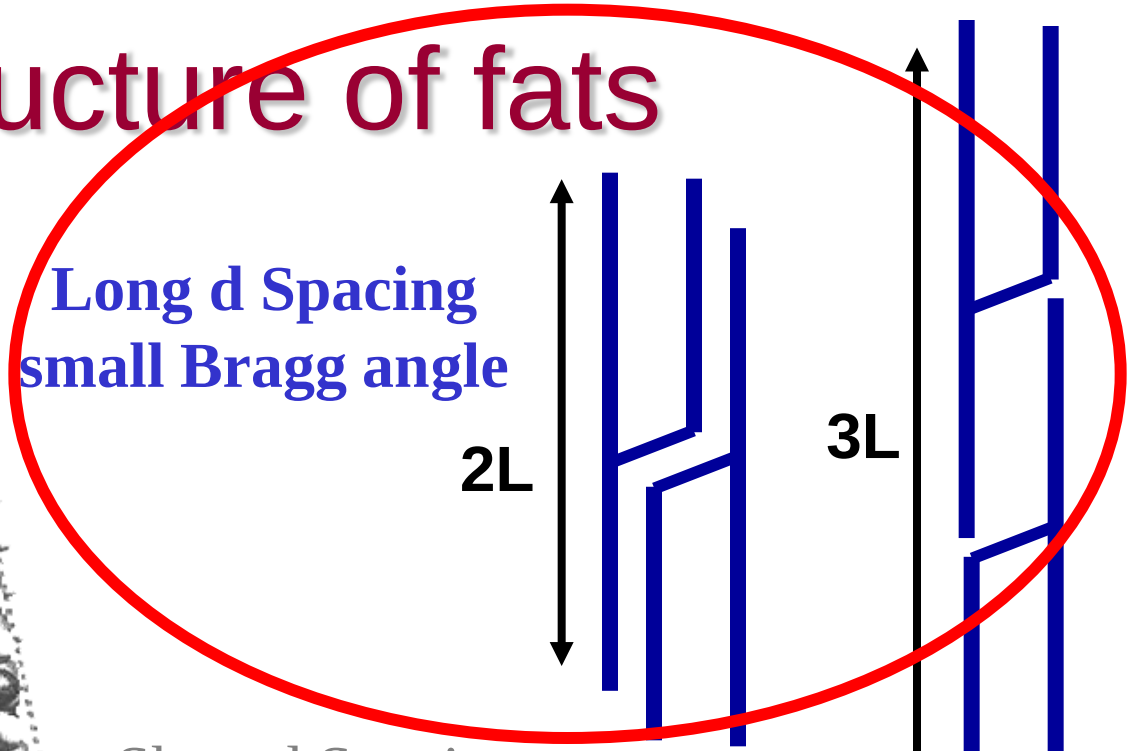
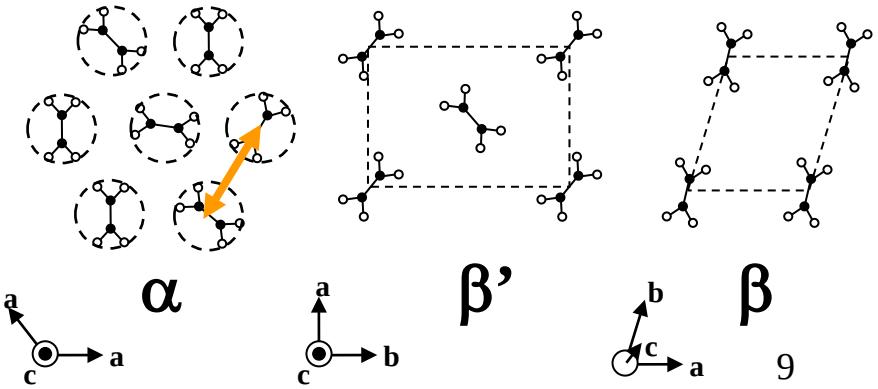
# Crystalline structure of fats




Short d Spacing
wide Bragg angle

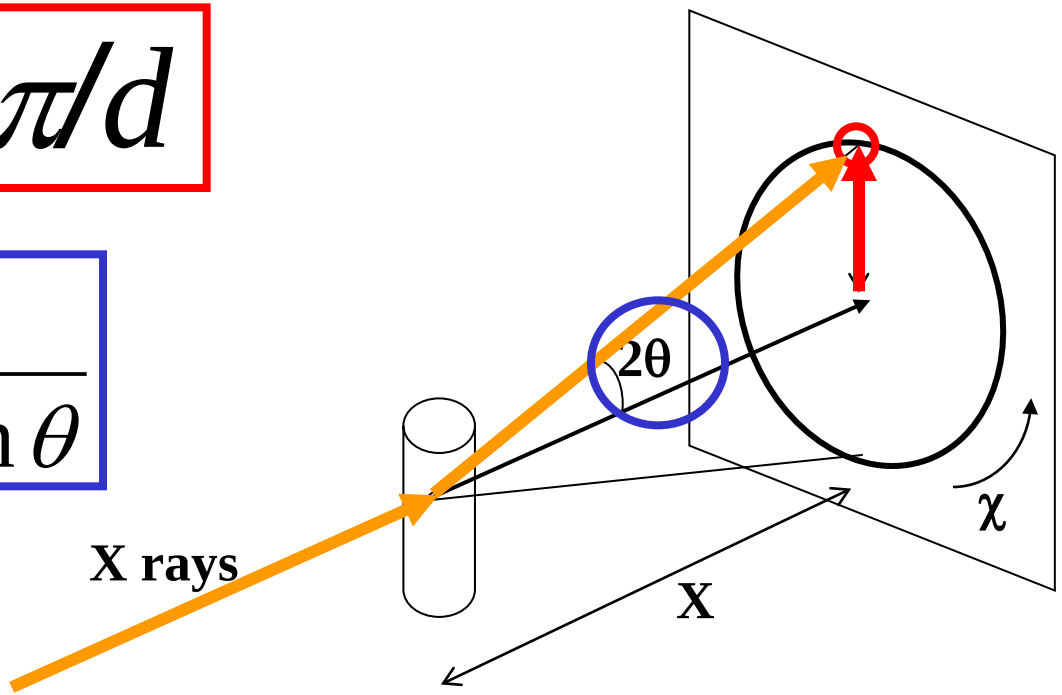



Modified from Garti & Sato: Crystallization Processes in Fats and Lipid Systems, 2nd ed.

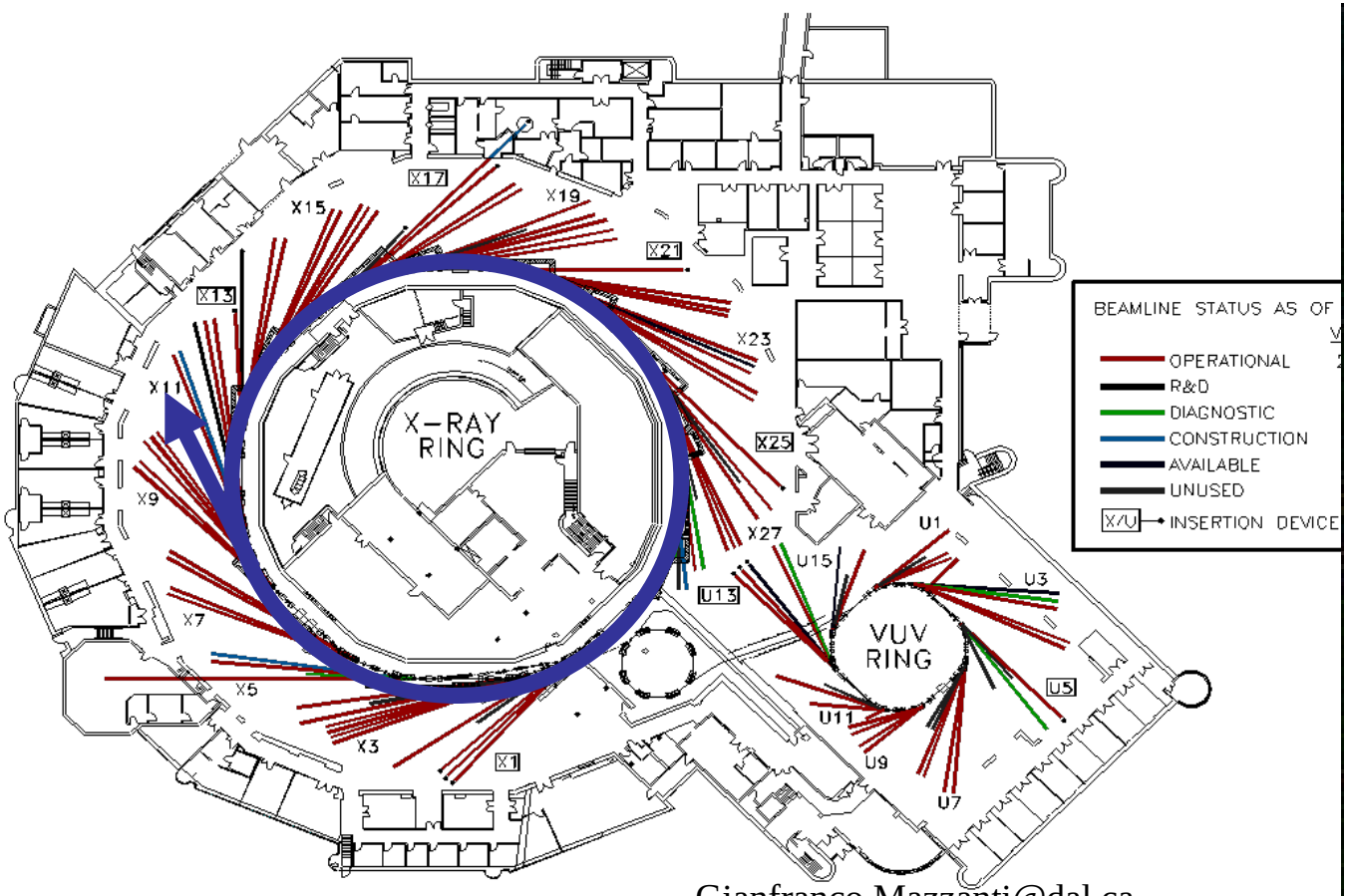
# 2 dimensional powder scattering

$$q = 2\pi/d$$

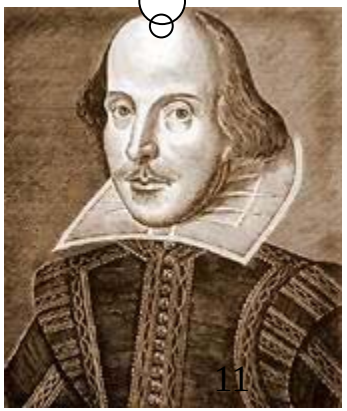
$$d = \frac{\lambda}{2\sin\theta}$$



# BNL-NSLS Synchrotron




Though this be madness yet there is method in't



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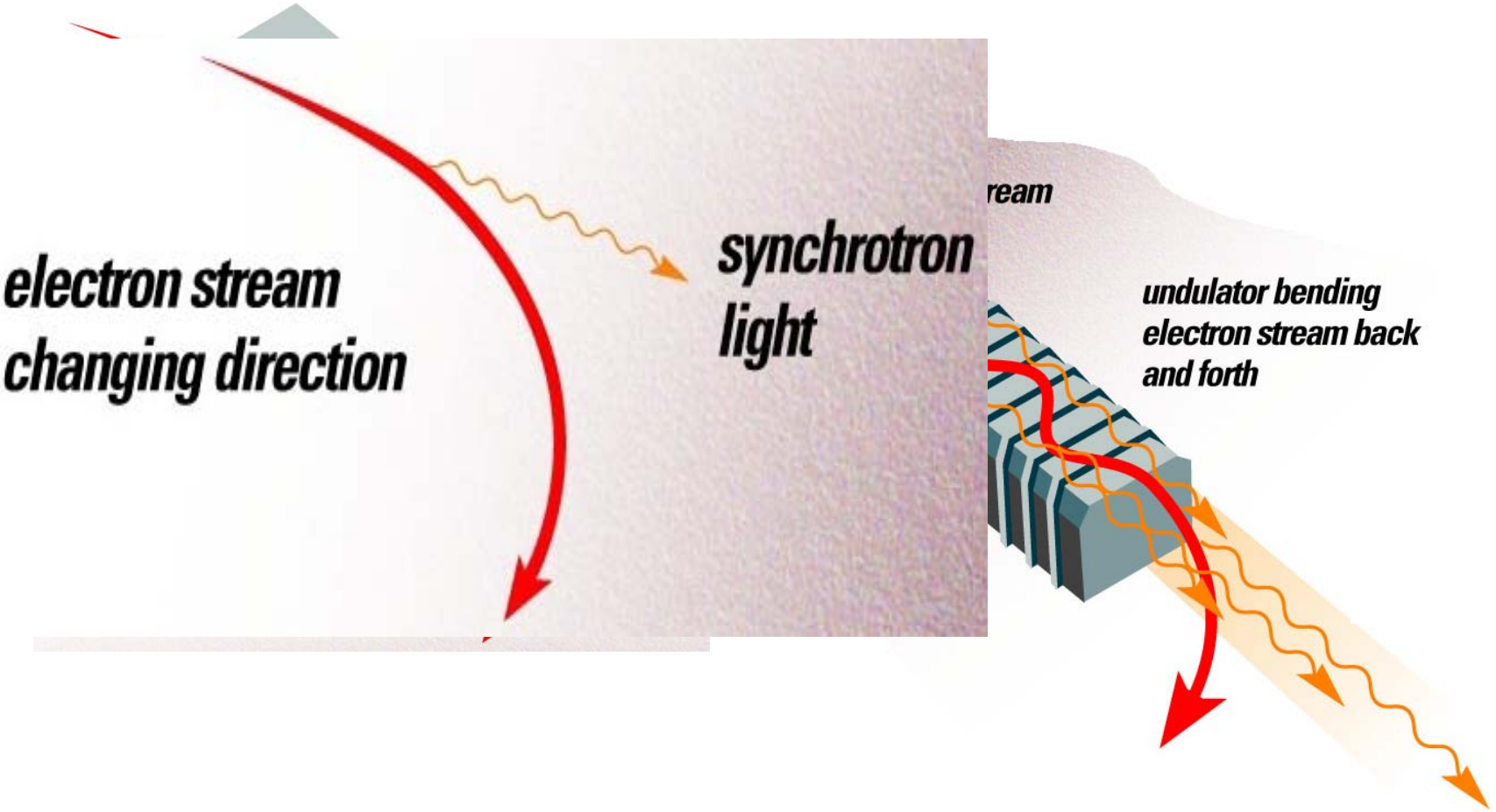
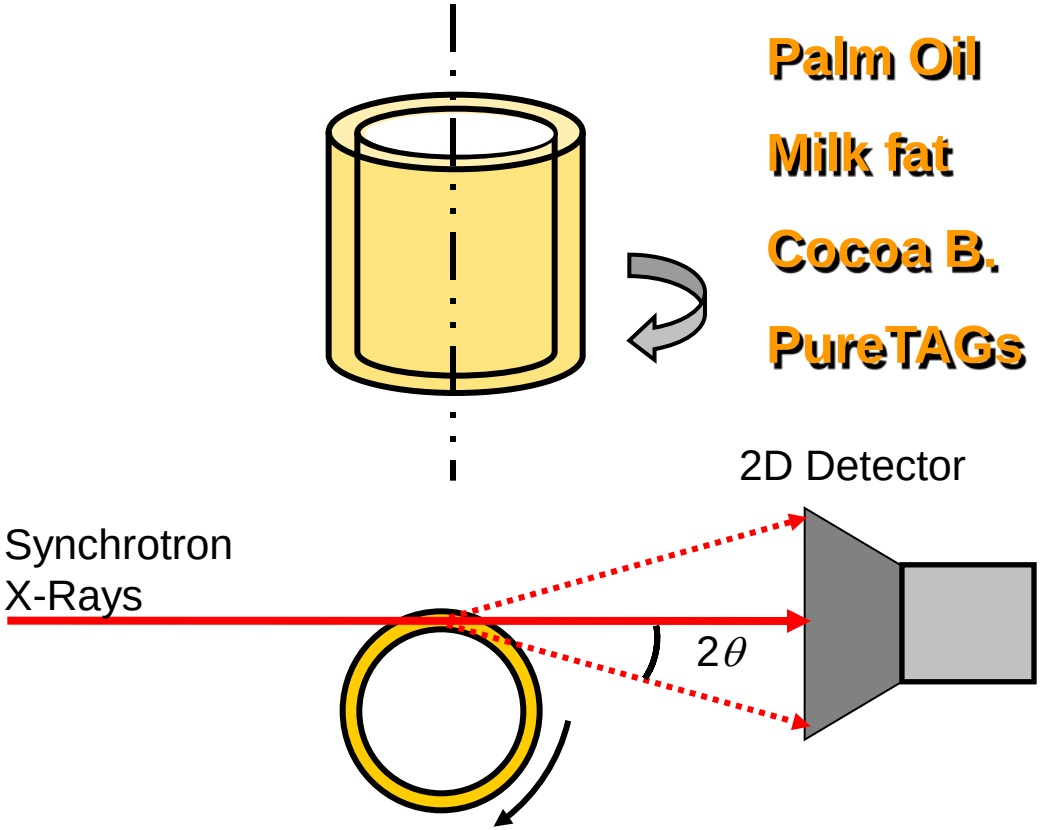
# How does it work?



# Experimental setup: Couette cell

50 ºC (30 min) ➔ 17 ~ 22 ºC at 3 ºC/min

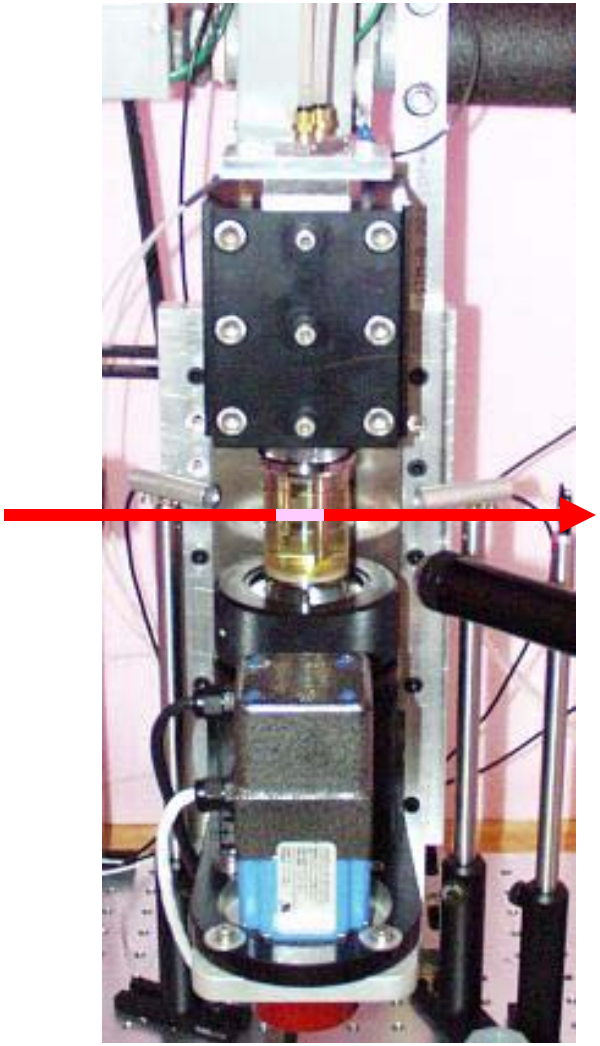



**Palm Oil**

**Milk fat**

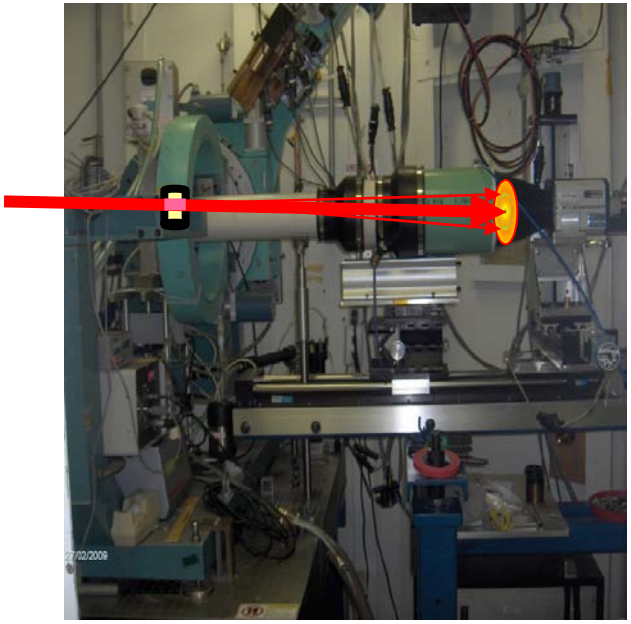
**Cocoa B.**

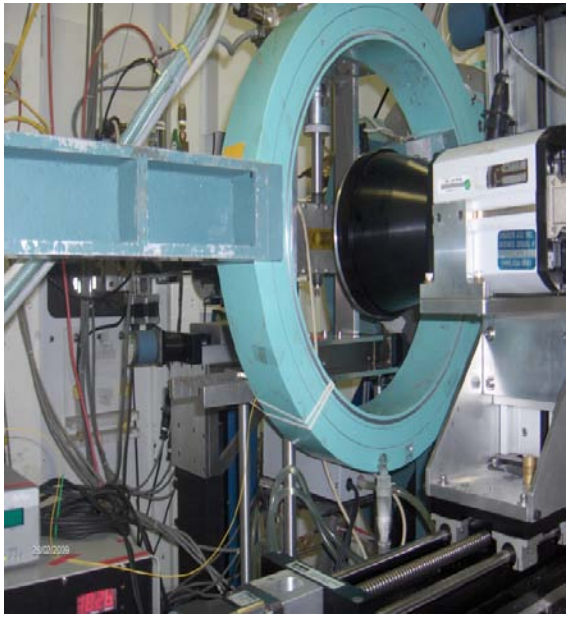
**PureTAGs**



$$\dot{\gamma} = \frac{\partial u}{\partial r} \approx \frac{w r_o}{r_o - r_i} \quad 0...2880\ s^{-1}$$

# Rheo-XRD at NSLS (BNL, Upton, NY)





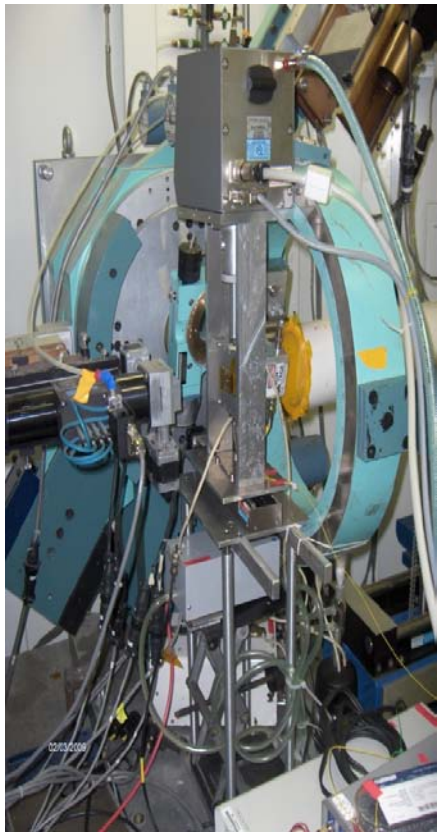
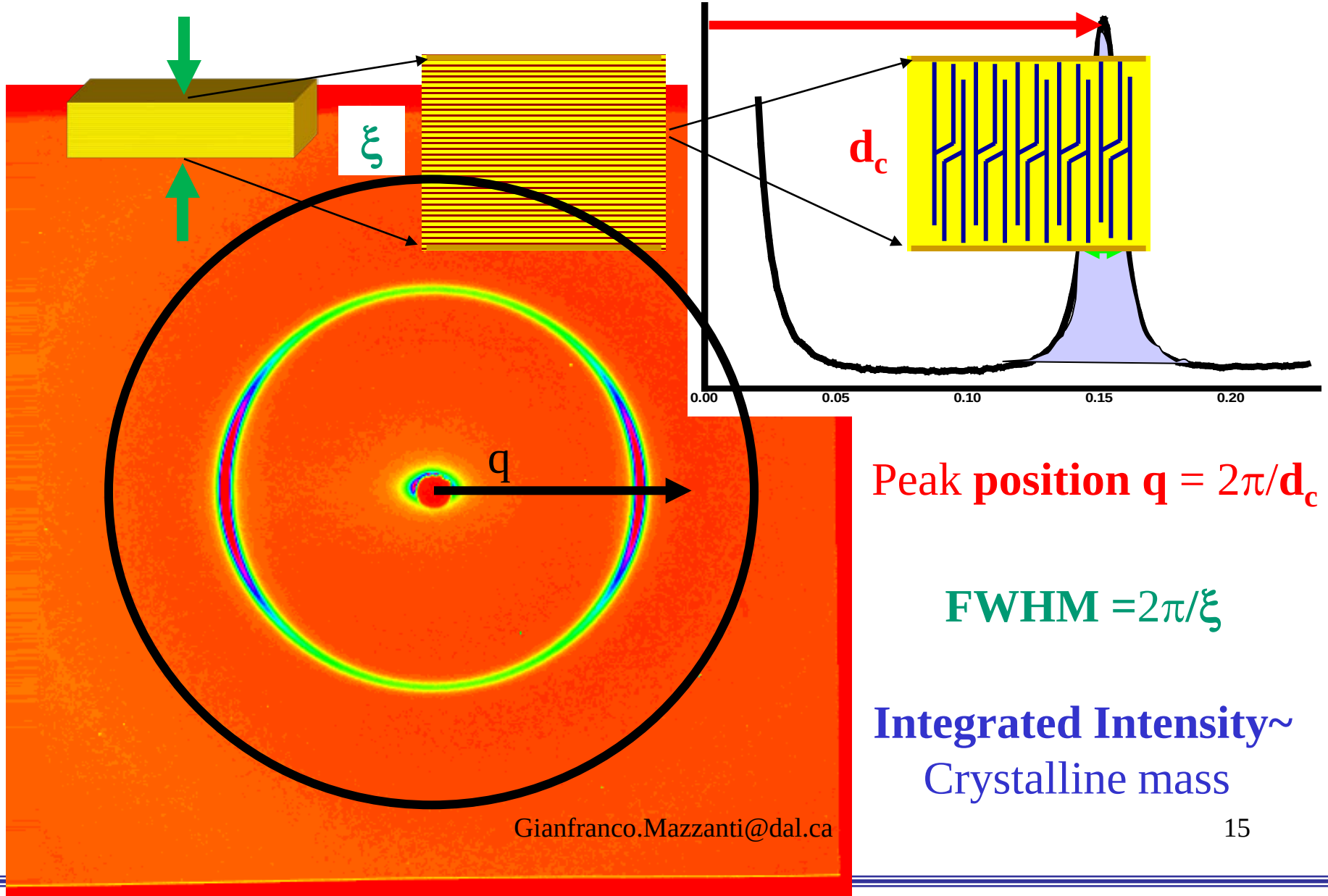


Fig. X-ray diffraction instrument

Fig. Shear & temperature control

# Phase characterization




Peak **position q** = $2\pi/\mathbf{d_c}$

**FWHM** $=2\pi/\xi$

**Integrated Intensity~**
Crystalline mass

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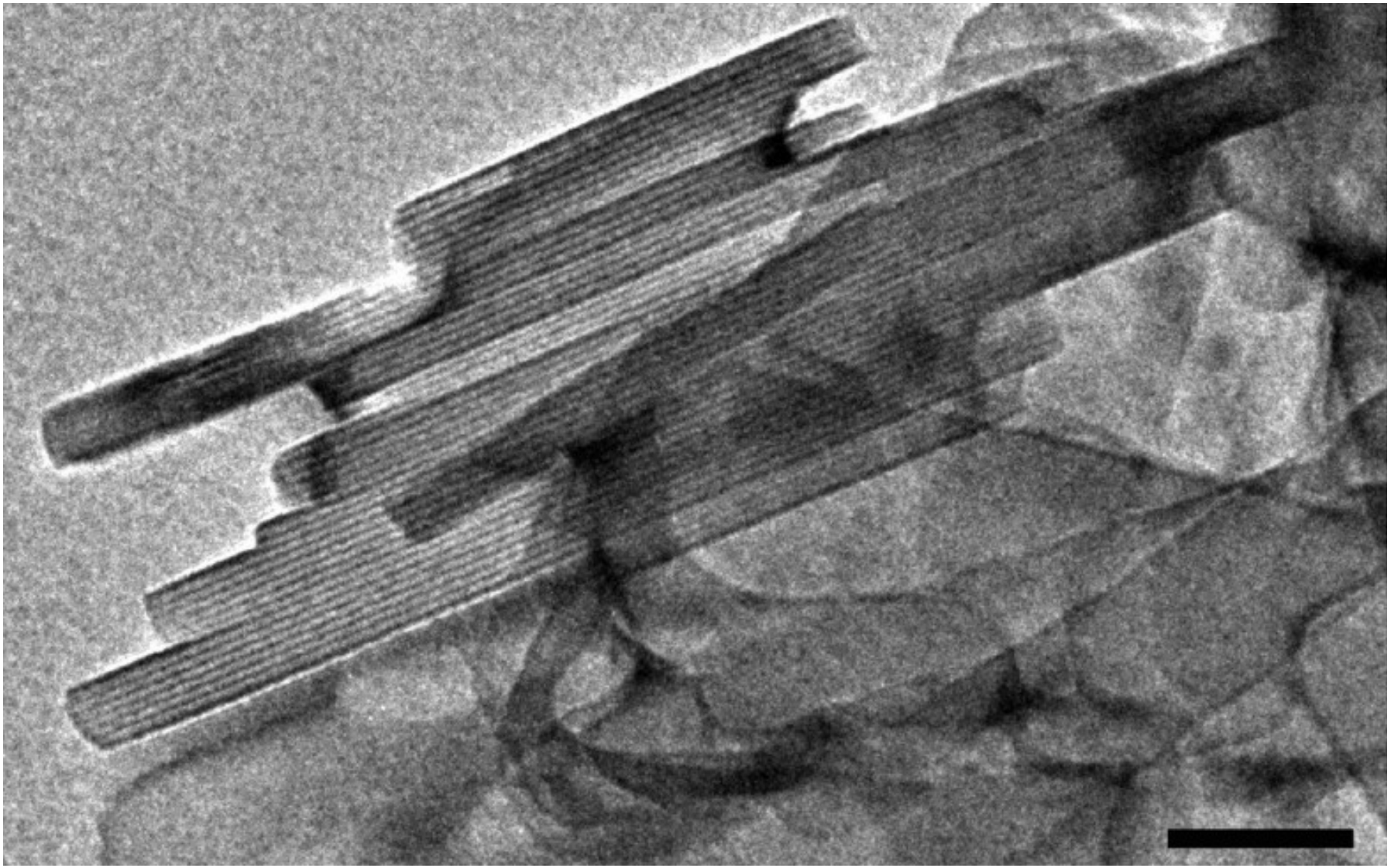


Acevedo& Marangoni, CGD, 2010

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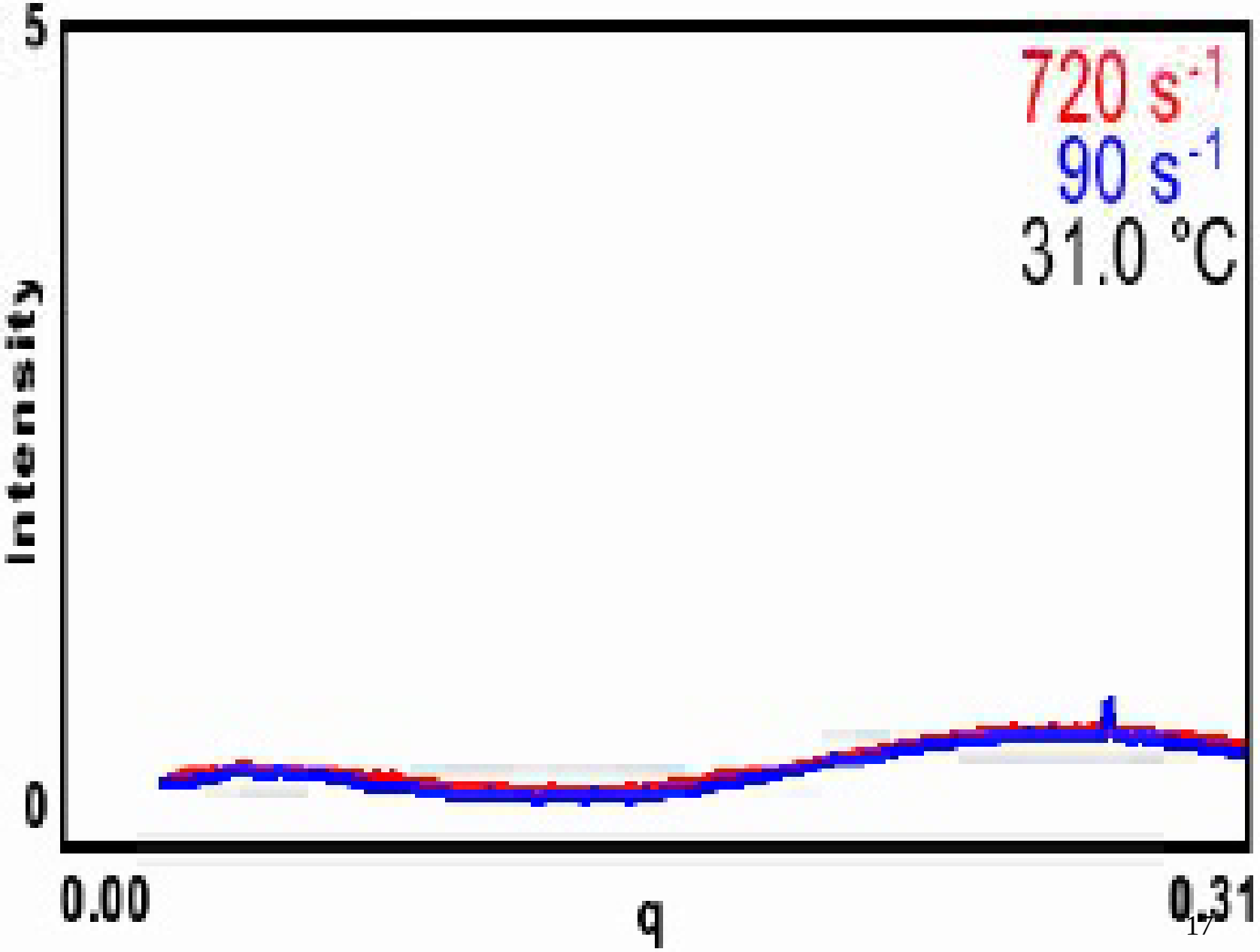
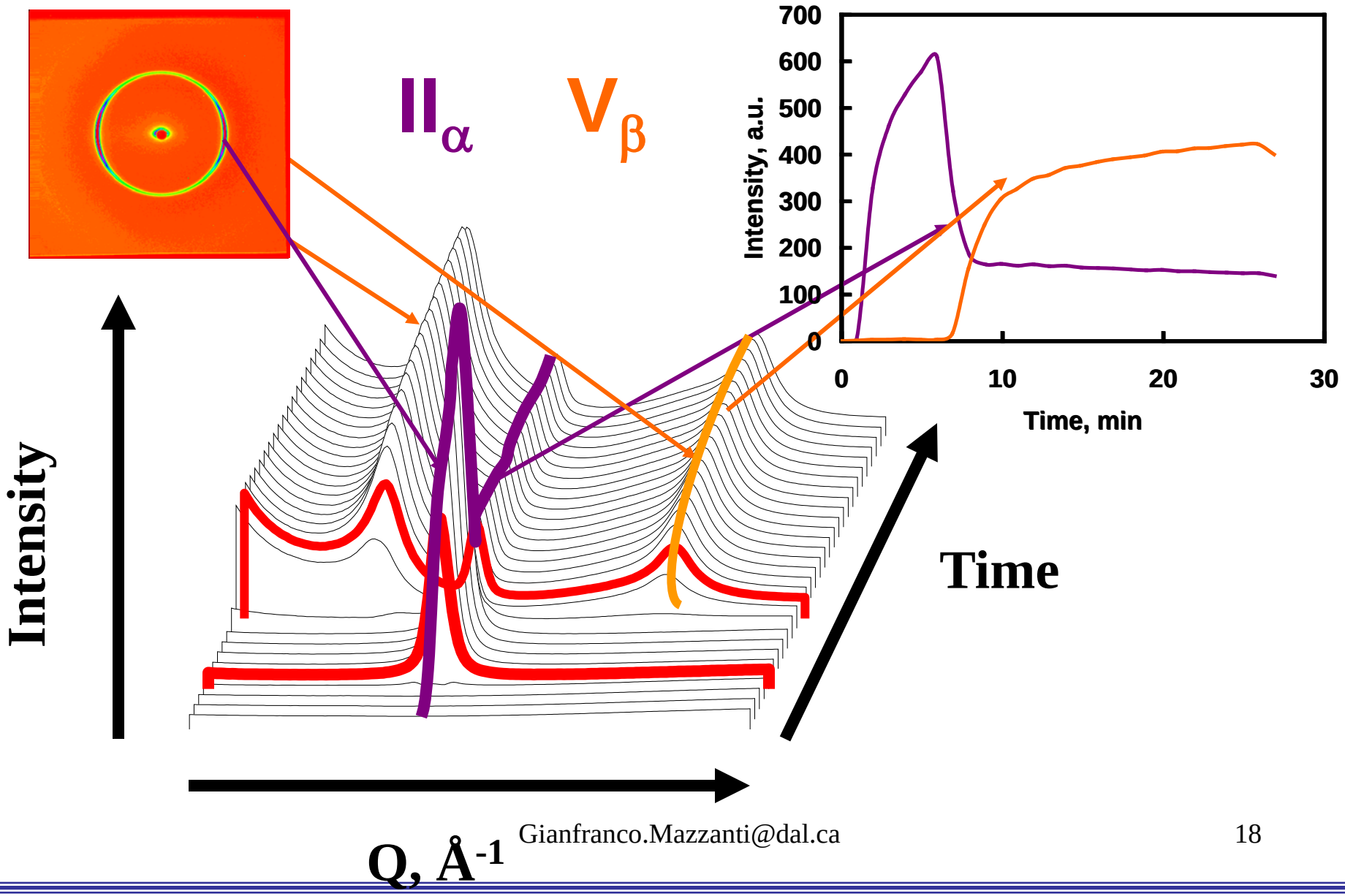
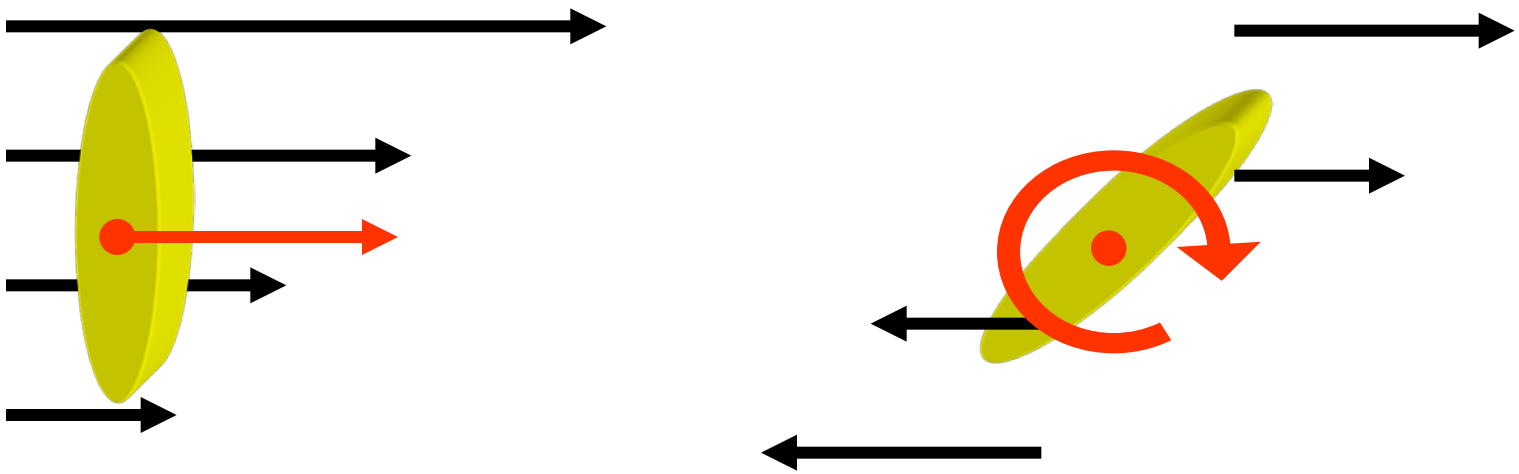

720 s-1
90 s-1
31.0 °C
5
0
Intensity
0.00
q
0.31

# Phase transitions



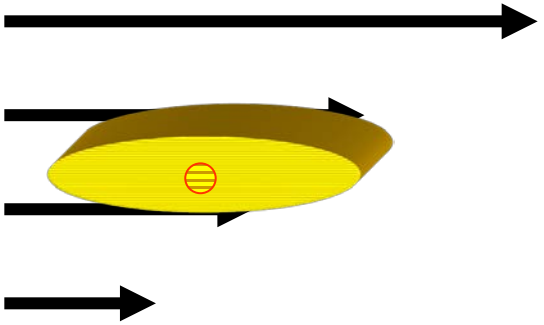
# Effect of shear on a nanoparticle

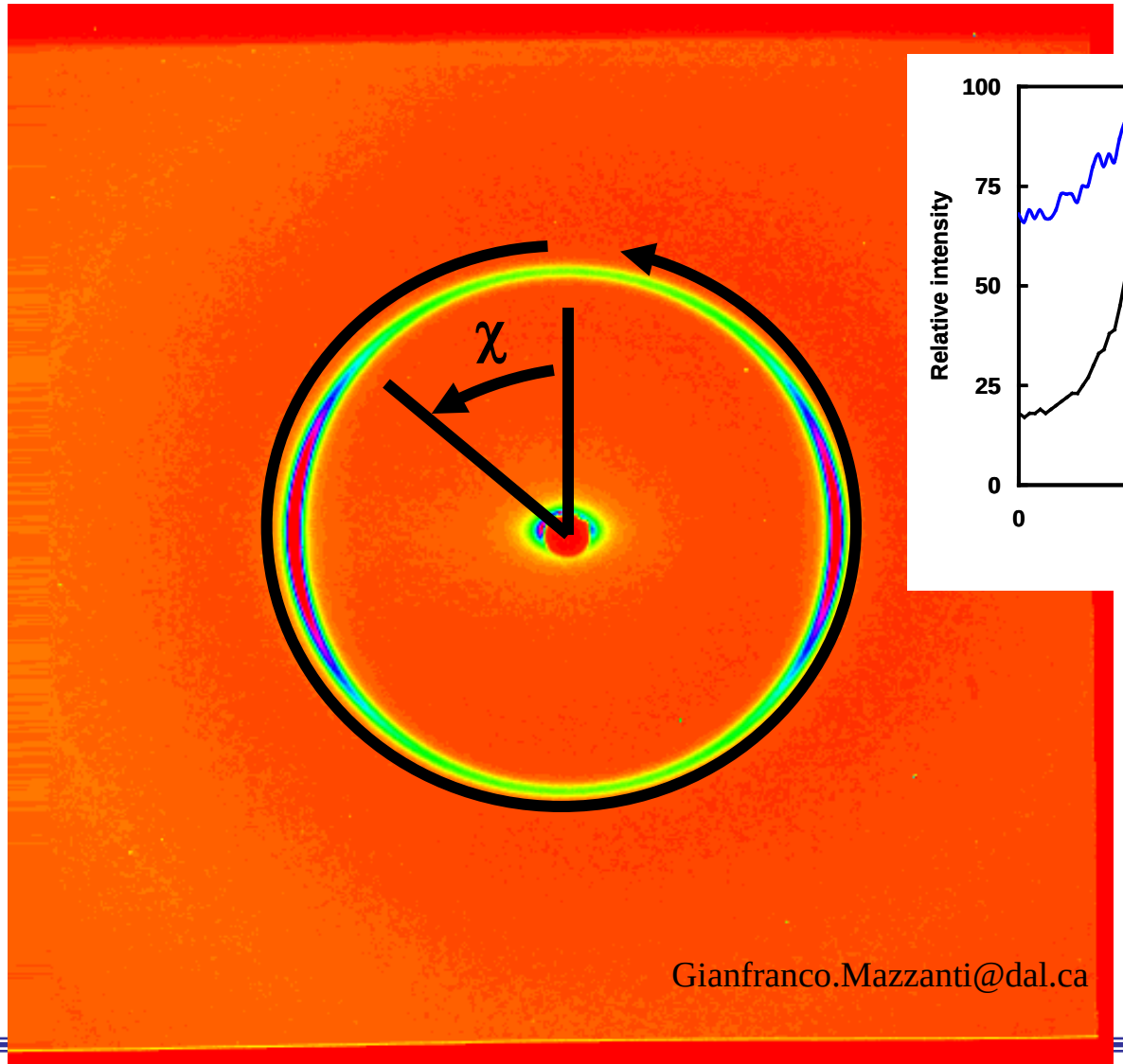
Shear = Velocity gradient

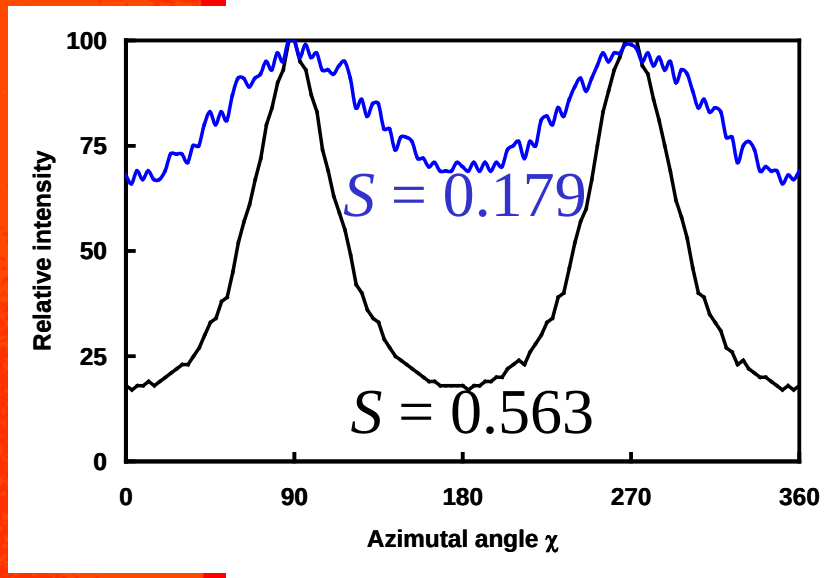


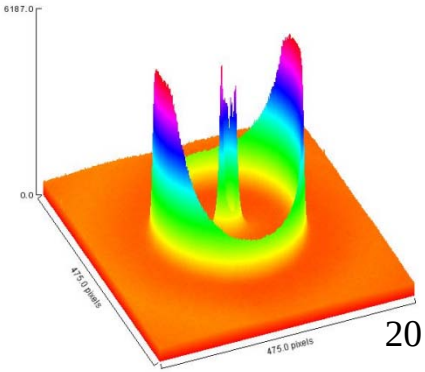
Shear induces rotation, so there may be preferred orientation with the long axis parallel to the shear lines, visible in oriented X-Ray diffraction patterns. Brownian movement, collisions & attraction oppose it.

# Orientation of crystals



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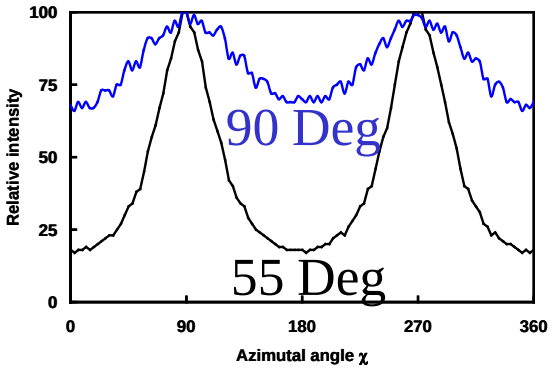
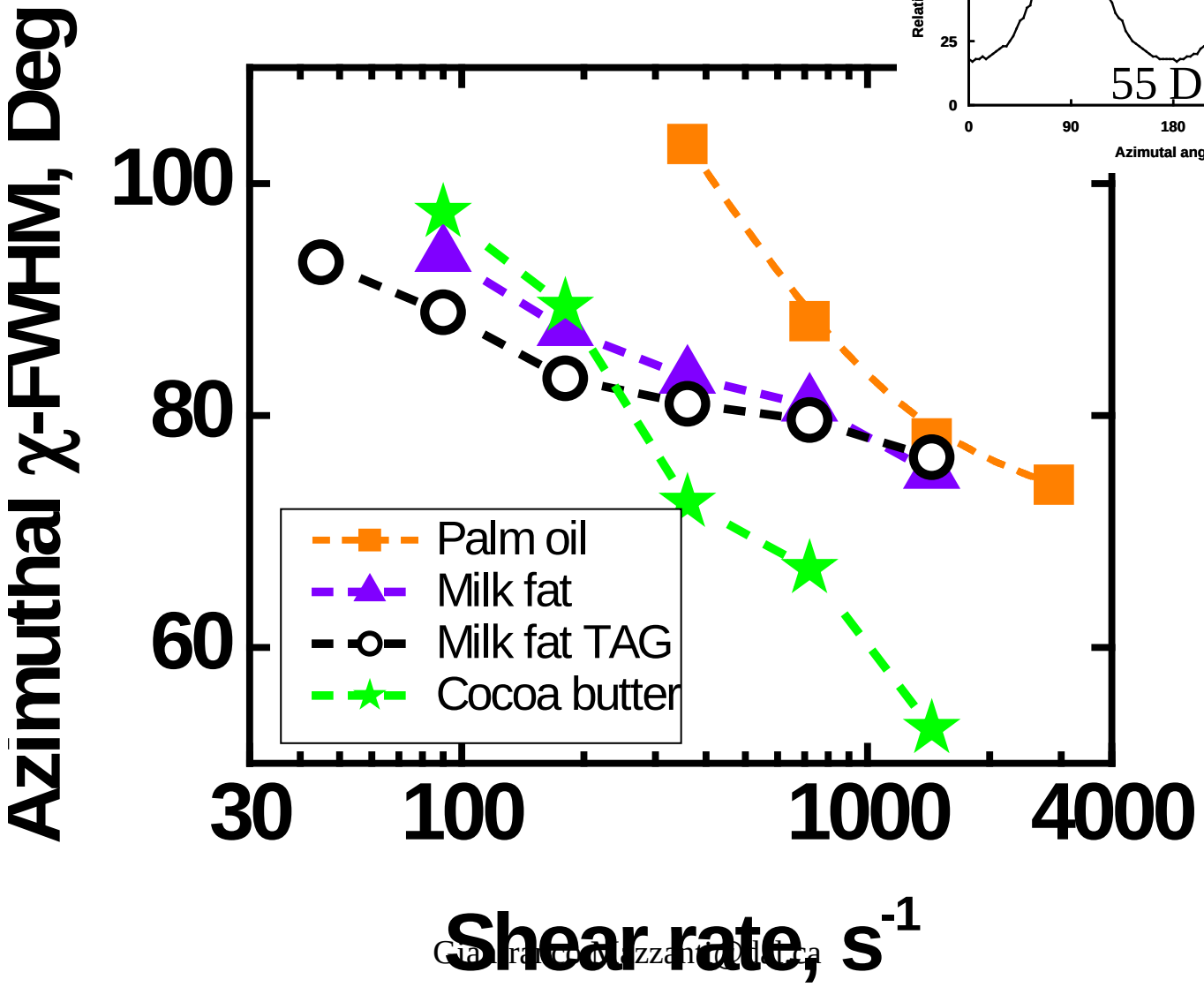
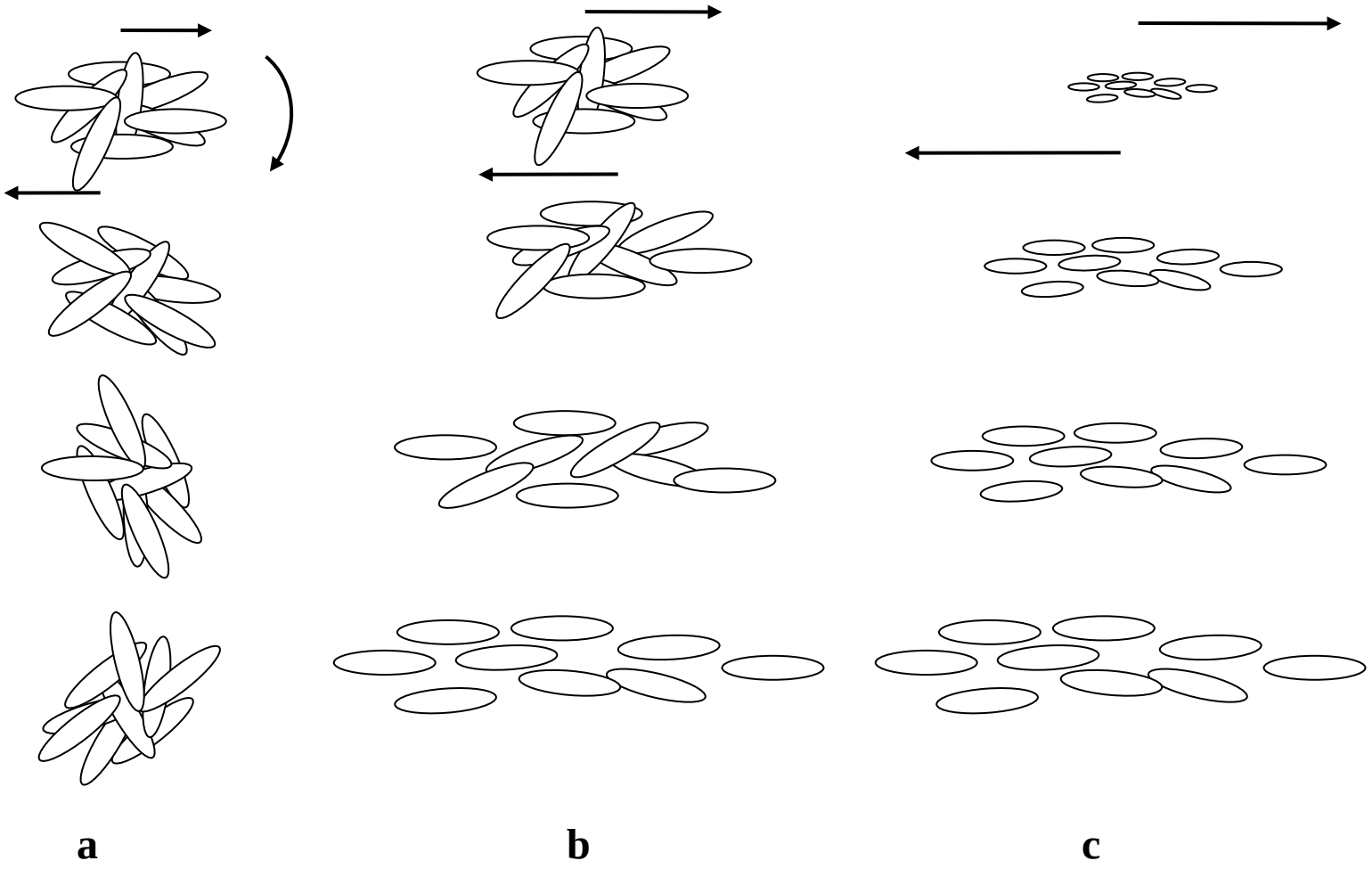
# Orientation distribution

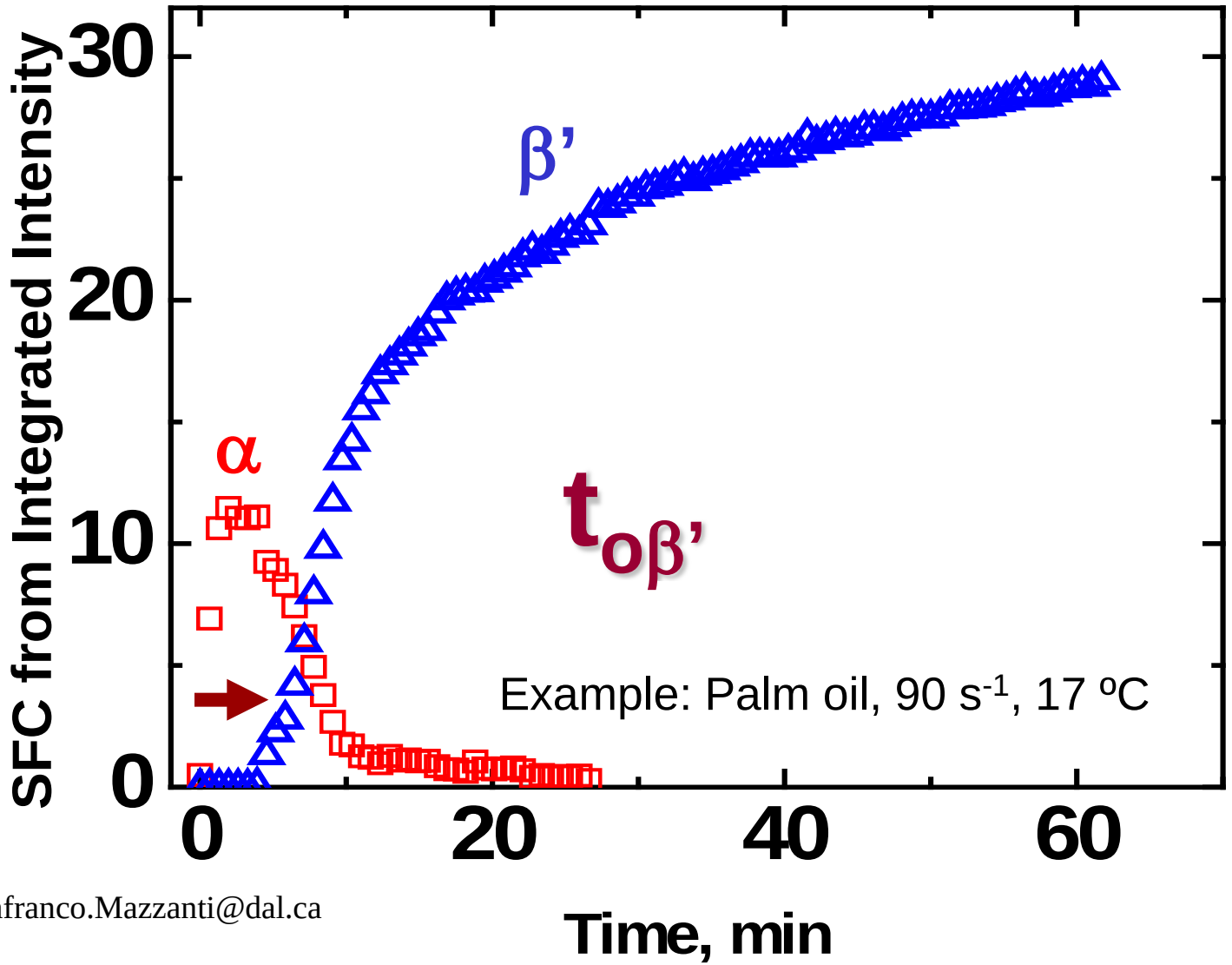


# Effect of shear on groups of particles



# Integrated Intensity

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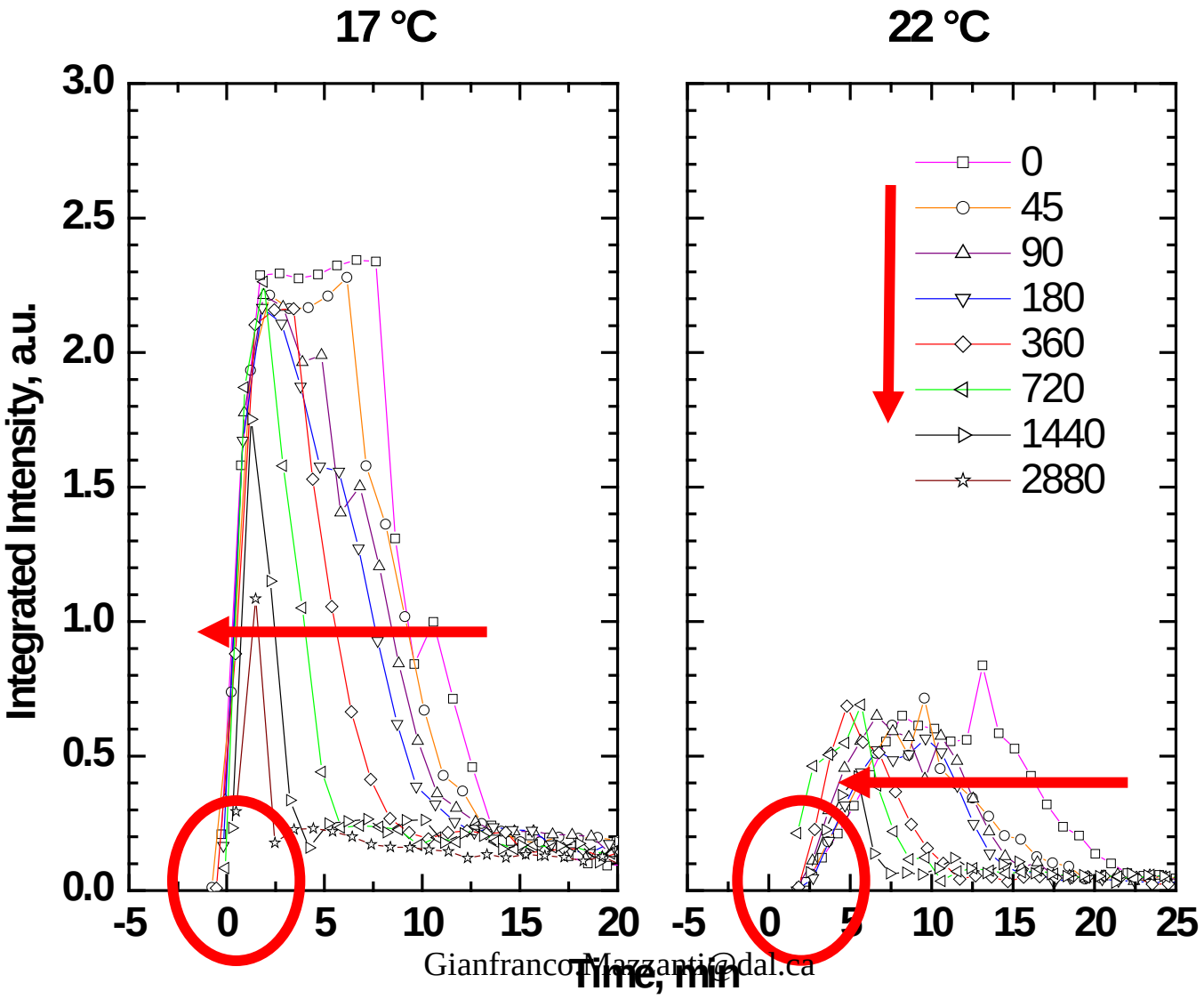
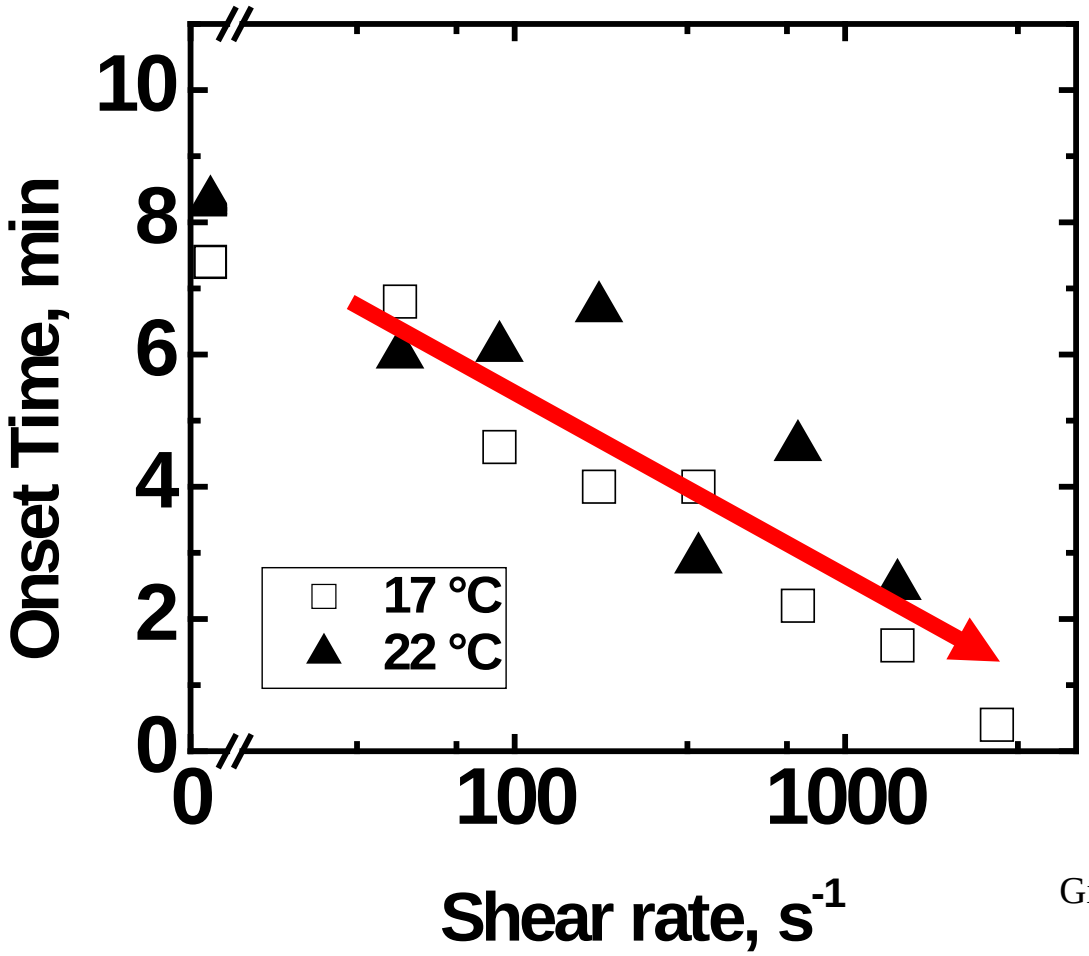
# Integrated intensity of phase α palm oil



# Onset time reduction - β' palm oil

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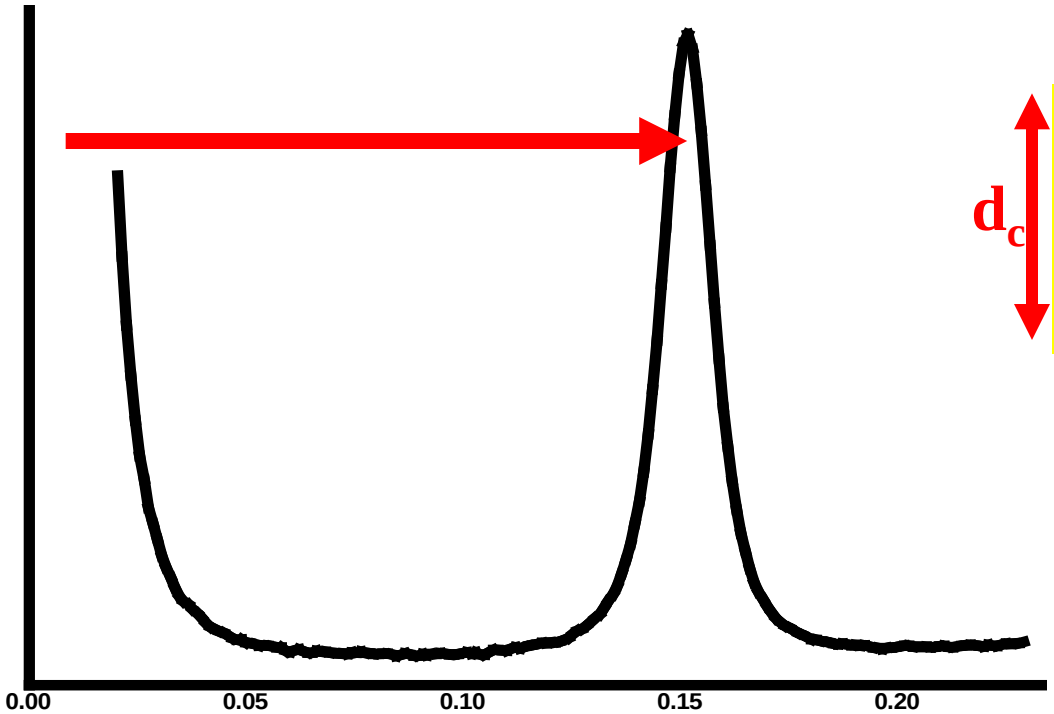
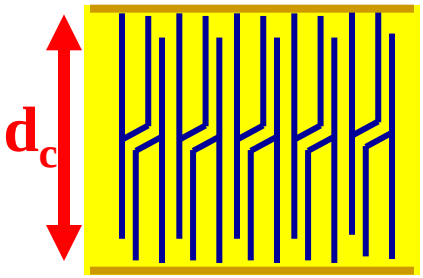
**GENERAL OBSERVATION FOR ALL FATS !**

# Peak position and composition

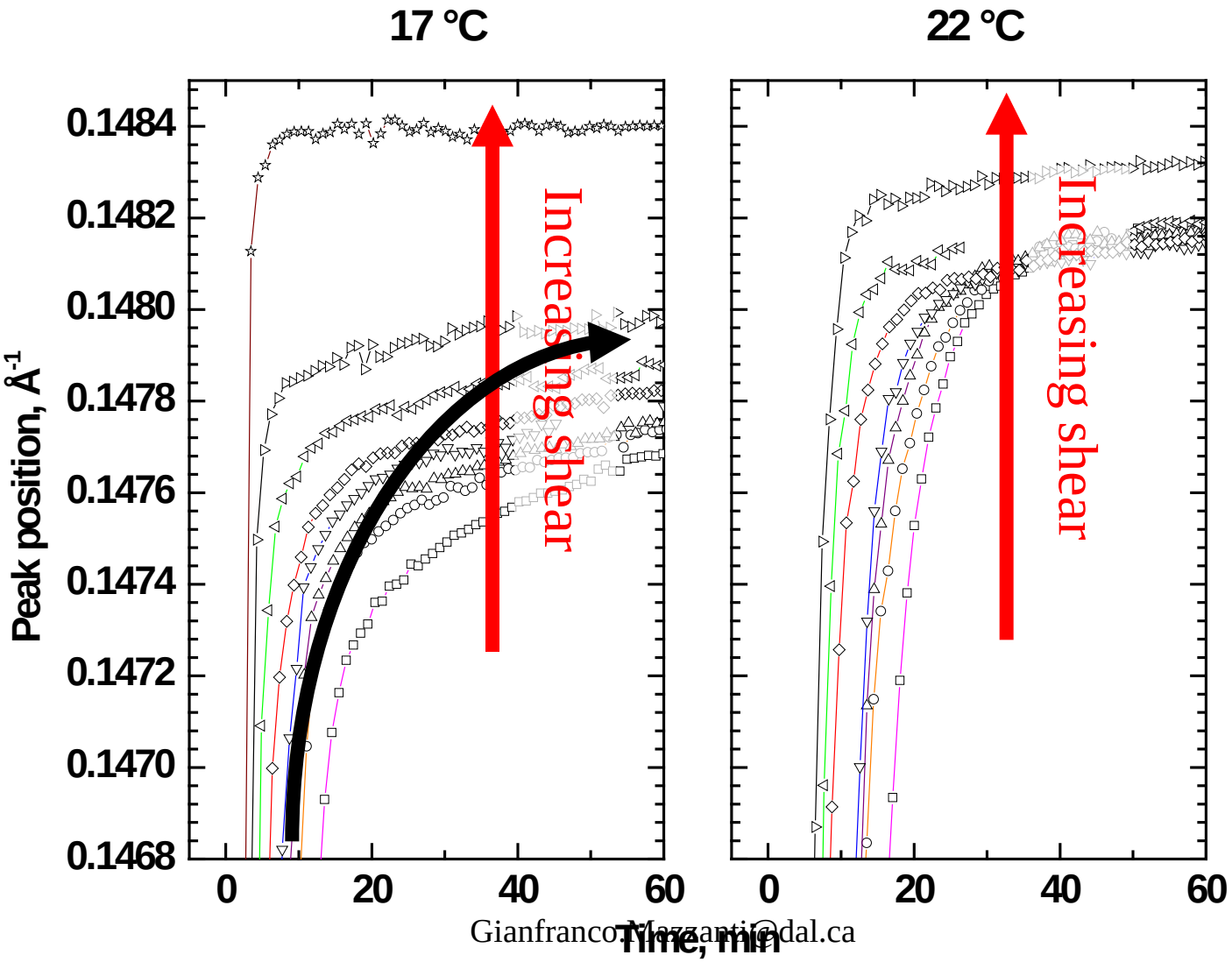


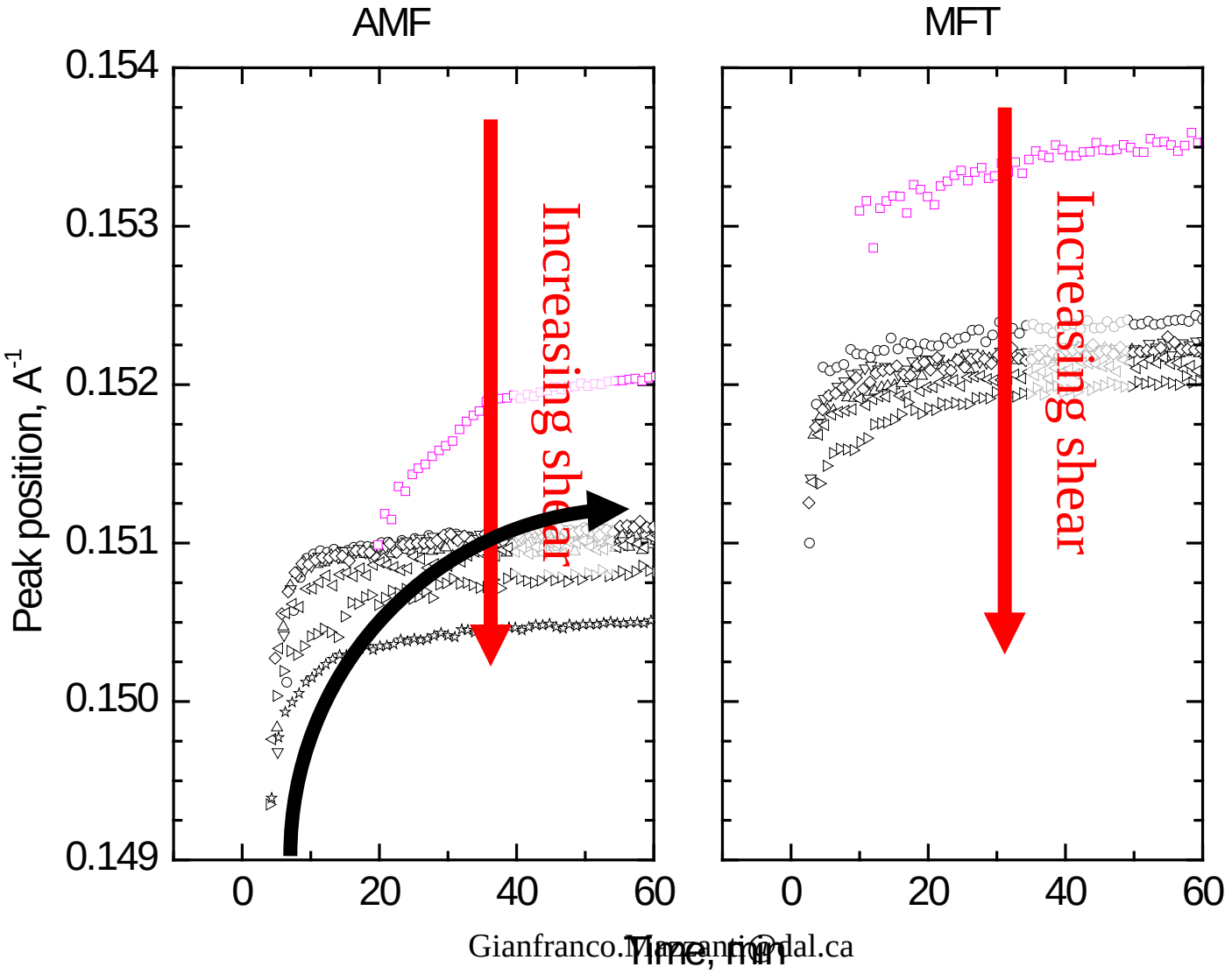

Peak **position**

$$\mathbf{q} = 2\pi/\mathbf{d_c}$$

# Peak position variation palm oil



# Peak position variation milk fat

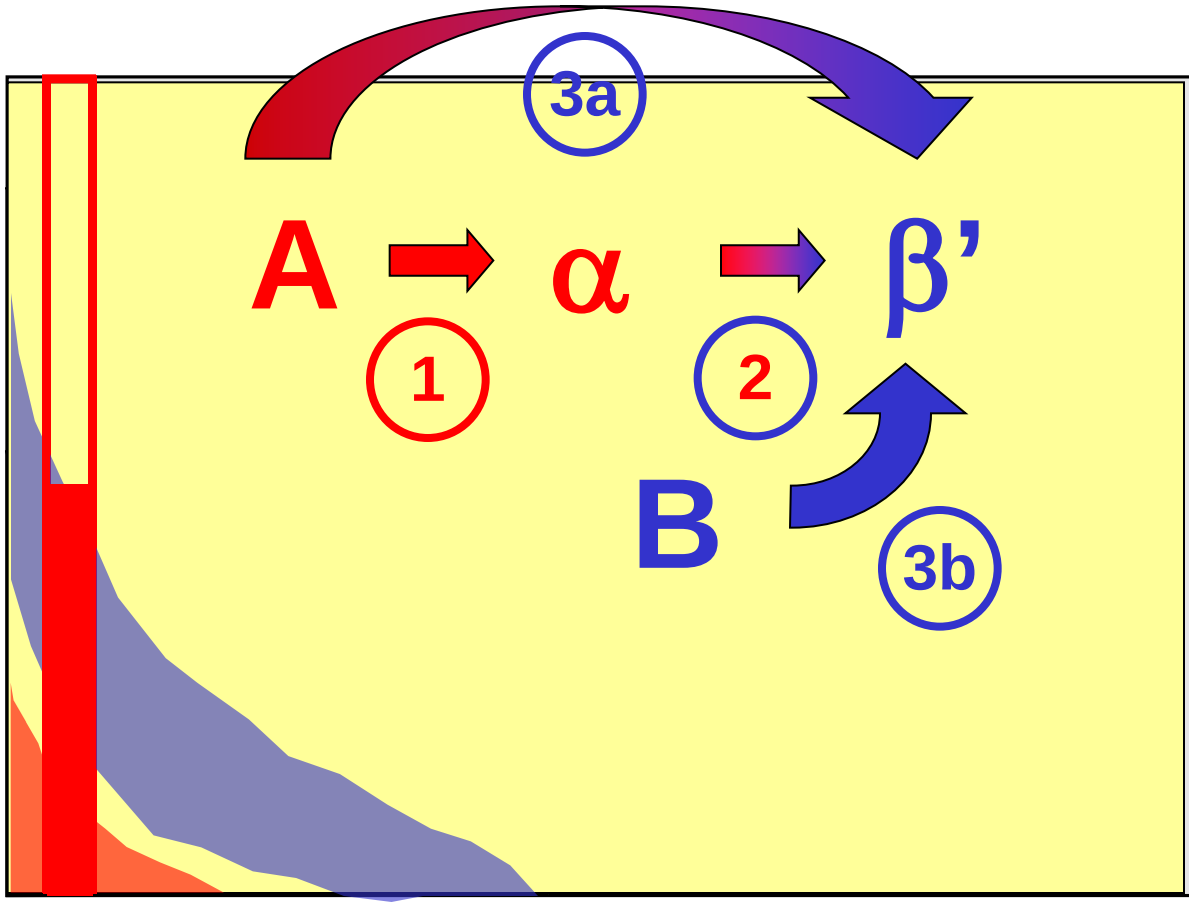


# Two crystallization compositional 'rules' from d-spacing changes

| | HMF | | MMF | | LMF | |
|---|---|---|---|---|---|---|
| | % | size | % | size | % | size |
| **Palm oil** | 11 | med/short | 46 | med/long | 43 | med/long |
| **Milk fat** | 20 | long | 10 | med | 70 | med/short |

- The longer molecules of a given fraction will tend to crystallize first, followed by the shorter ones of that fraction.
- The proportion of molecules of the **HMF** mixed with MMF+LMF increases with temperature or shear.

# Kinetics of Phase growth



# Differential form of Avrami equation

$$\frac{\partial A}{\partial t} = -n_A \cdot k_A \cdot A \cdot \left[ -\ln\left( \frac{A}{A^*} \right) \right]^{\frac{n_A - 1}{n_A}}$$

$A$ **Amount of material yet to be transformed**

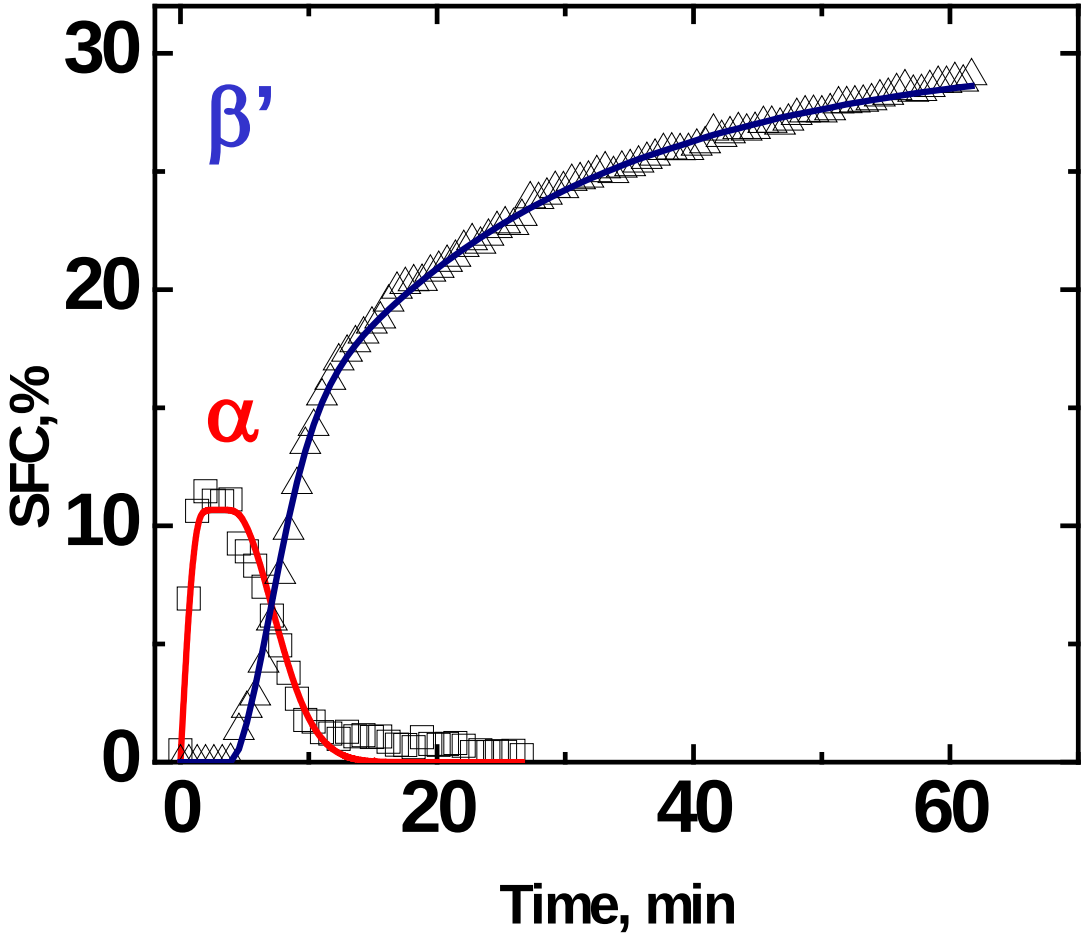
$A^*$ **Maximum amount of available material to transform**

$n_A$ **Growth mode exponent**

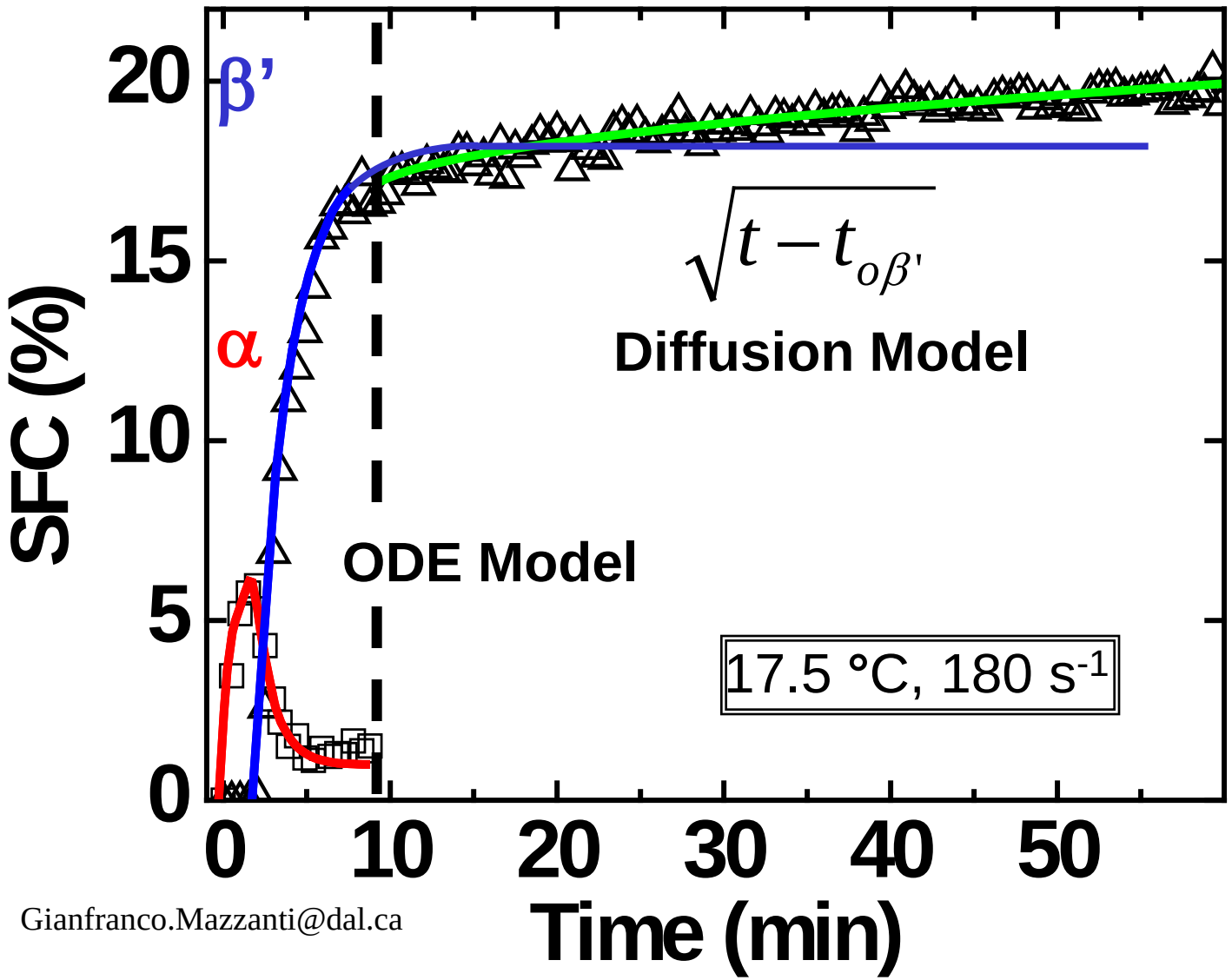
$k_A$ **Time scaling constant**

# Data vs. model

Example: Palm oil, 90 $s^{-1}$, 17 ºC



# Milk fat, a step closer to equilibrium

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# Summary of knowledge

- Functions of composition and temperature-shear profile to estimate:
  - Characteristic lamellar size
  - Characteristic nanoplatelet sizes
  - Characteristic orientation related to particle segregation
  - Approximate amounts of solids formed under different growth regimes

2. Complementing the XRD findings for better quantification

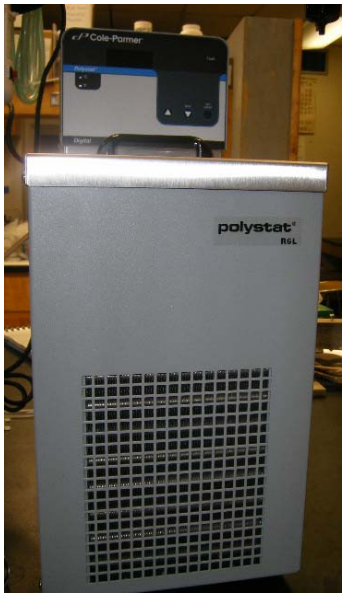
# Use of NMR & Rheology under shear flow

# Experimental Methods

**Rheometer**

**(Anton Paar Co. DSR 301)**

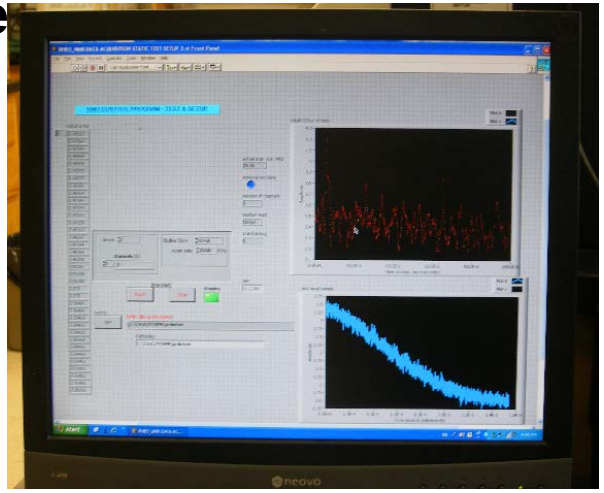
**Fat sample**



**Temperature Control Syste**

+

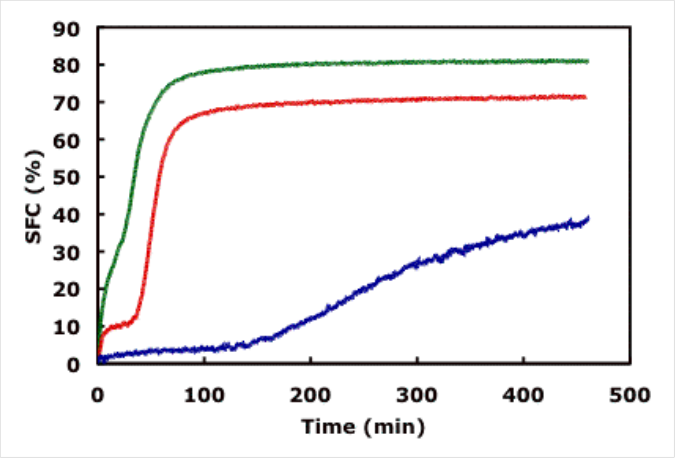
**NMR (IBM PC/20)**

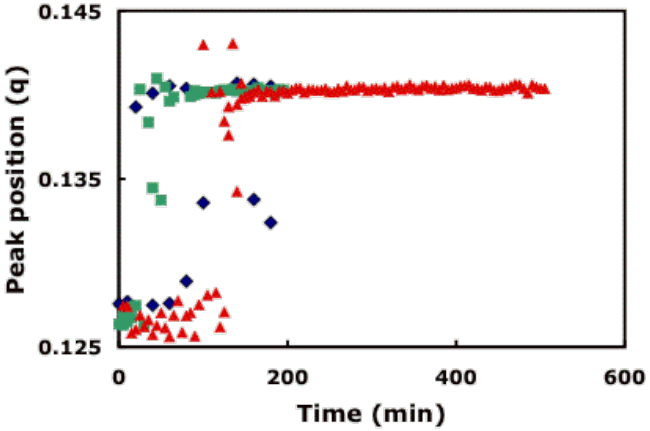


**Computer programs**

# Results: static

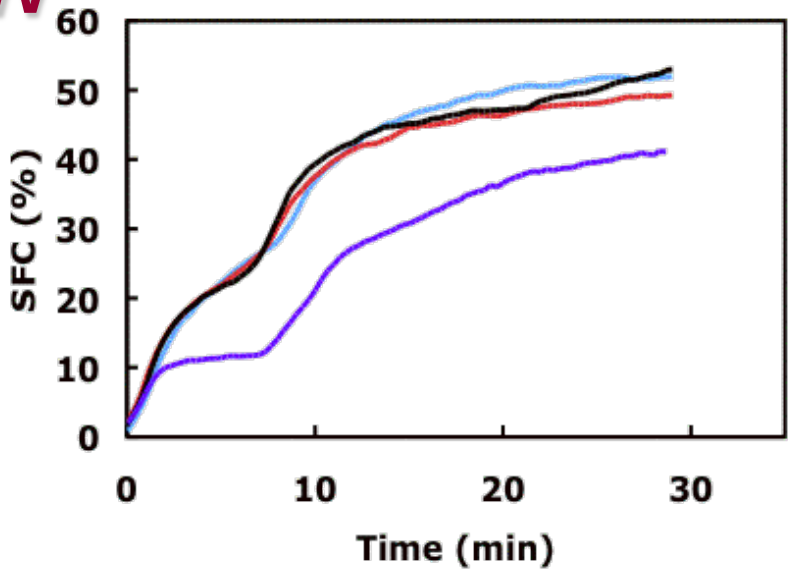
- Static
  - Resembled previous research
  - SFC dependent on Temperature
  - Increased Temp = prolonged Transitions
  - Form II to IV

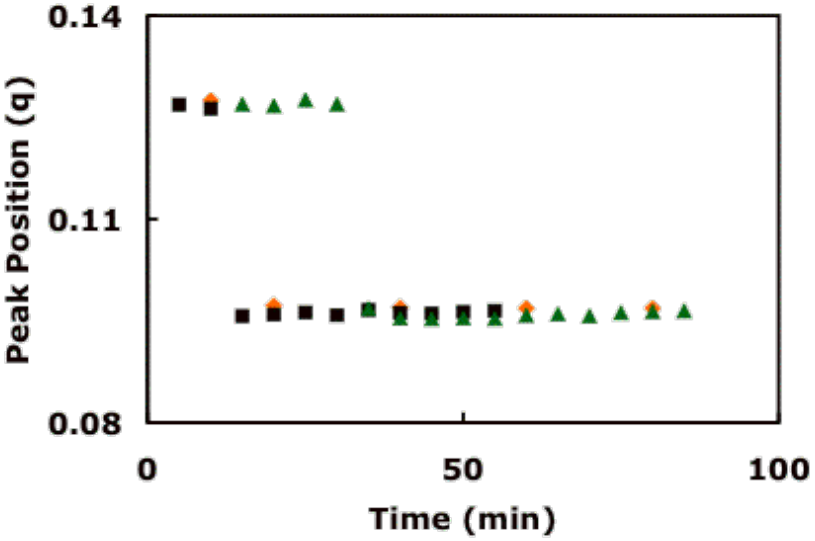


# Results: shear flow

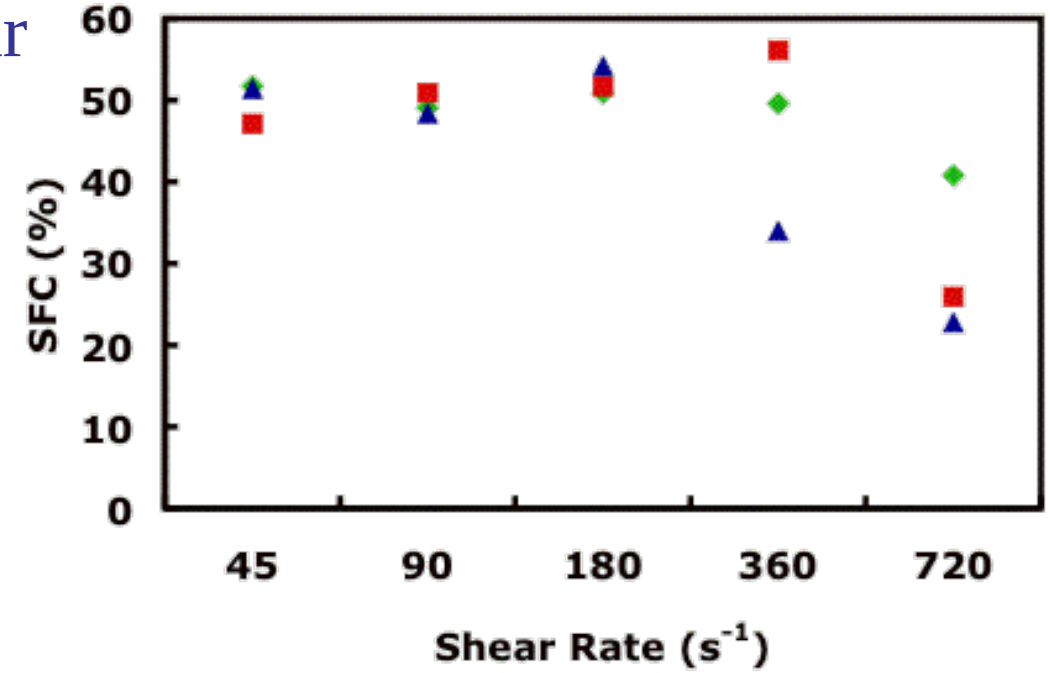
- E.g. 17.5 °C
  - SFC remained around 50%
  - Form II to V, no form IV
  - 720 $s^{-1}$ lower SFC
    - Viscous heating



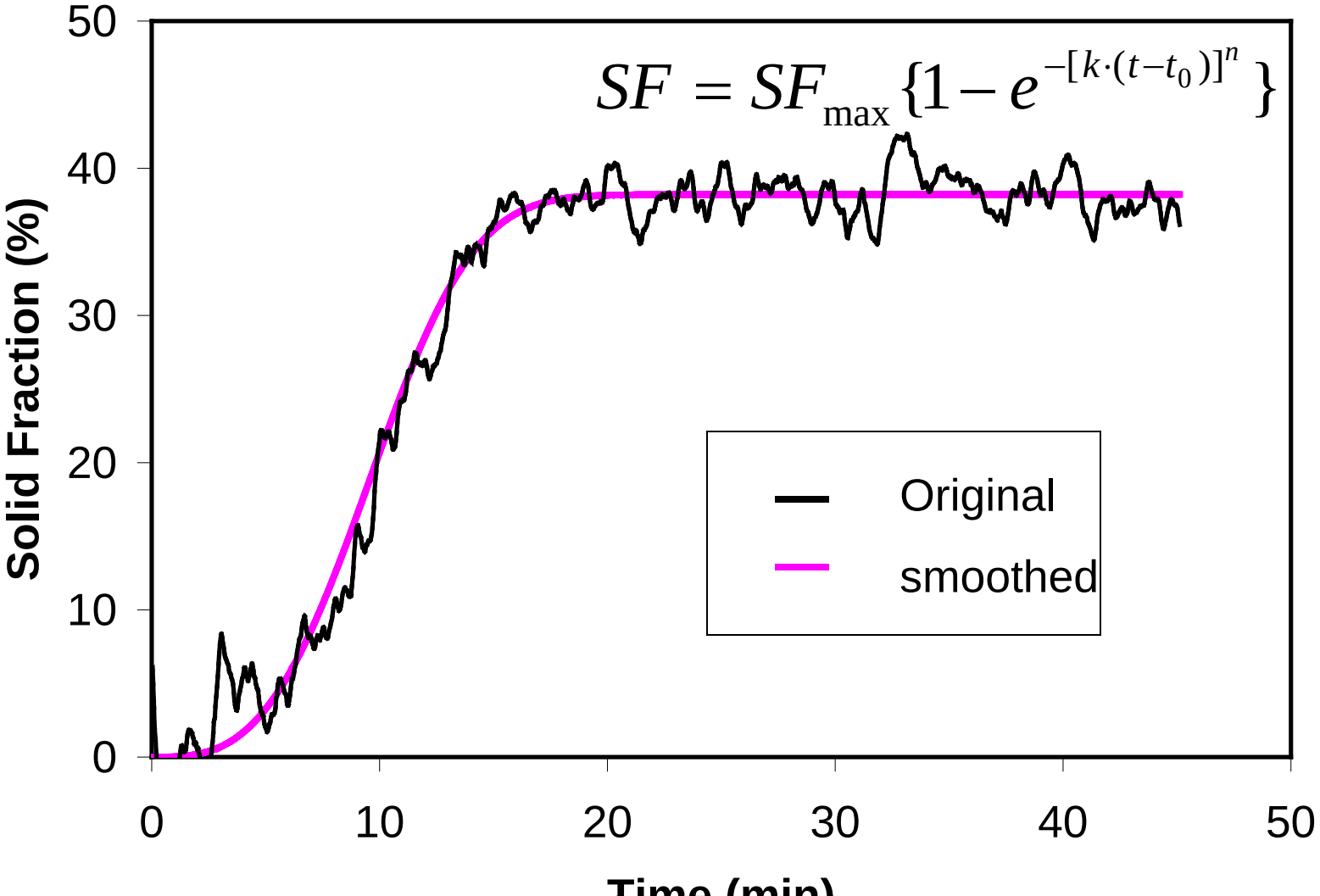



# Results summary

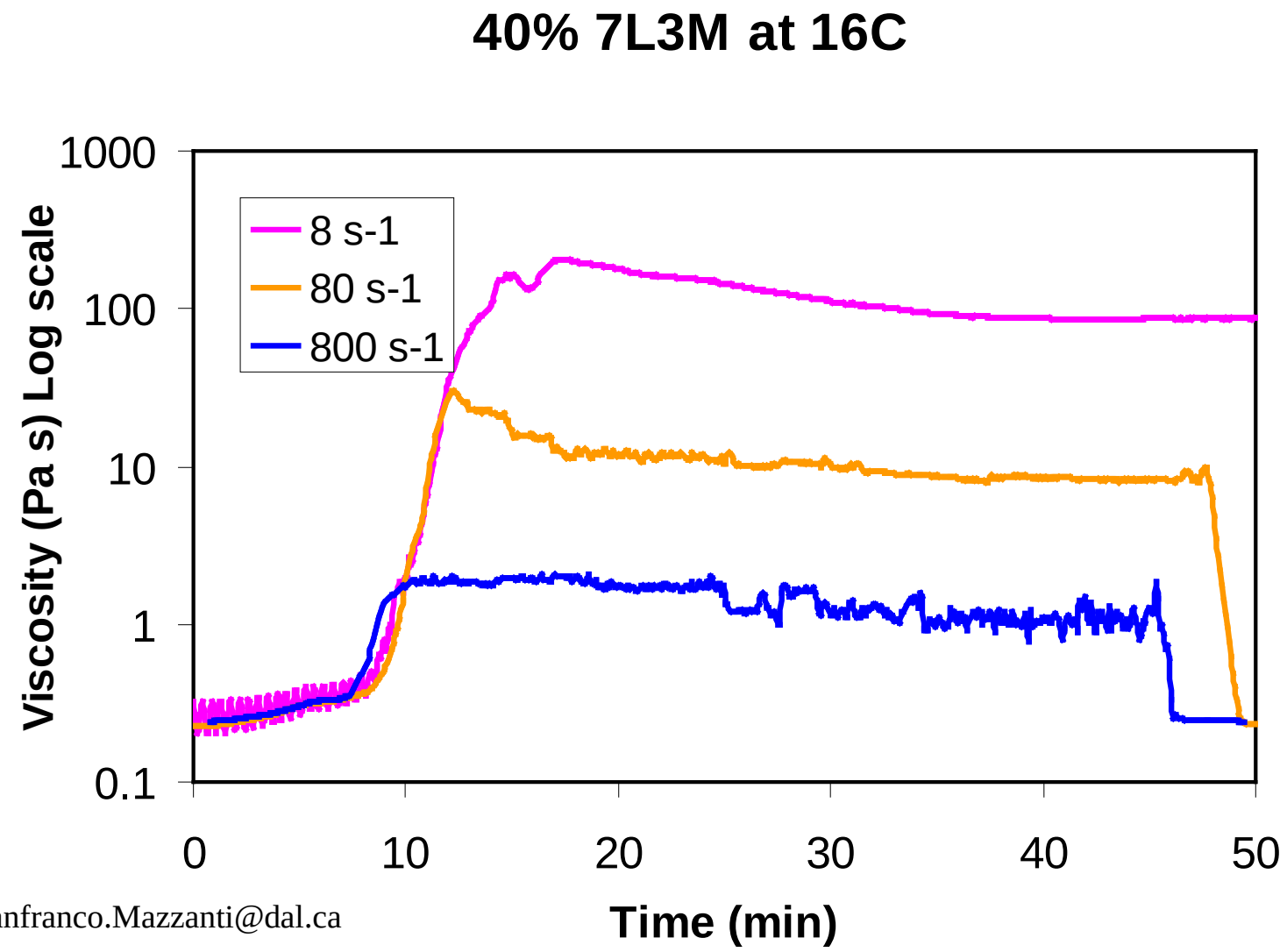
- When form V present (lower temp and shear rate)
  - Max SFC around 50%
  - Not dependent on temperature
    - Outside melting range



# Smoothing Model (Avrami)



# Results-Rheometer

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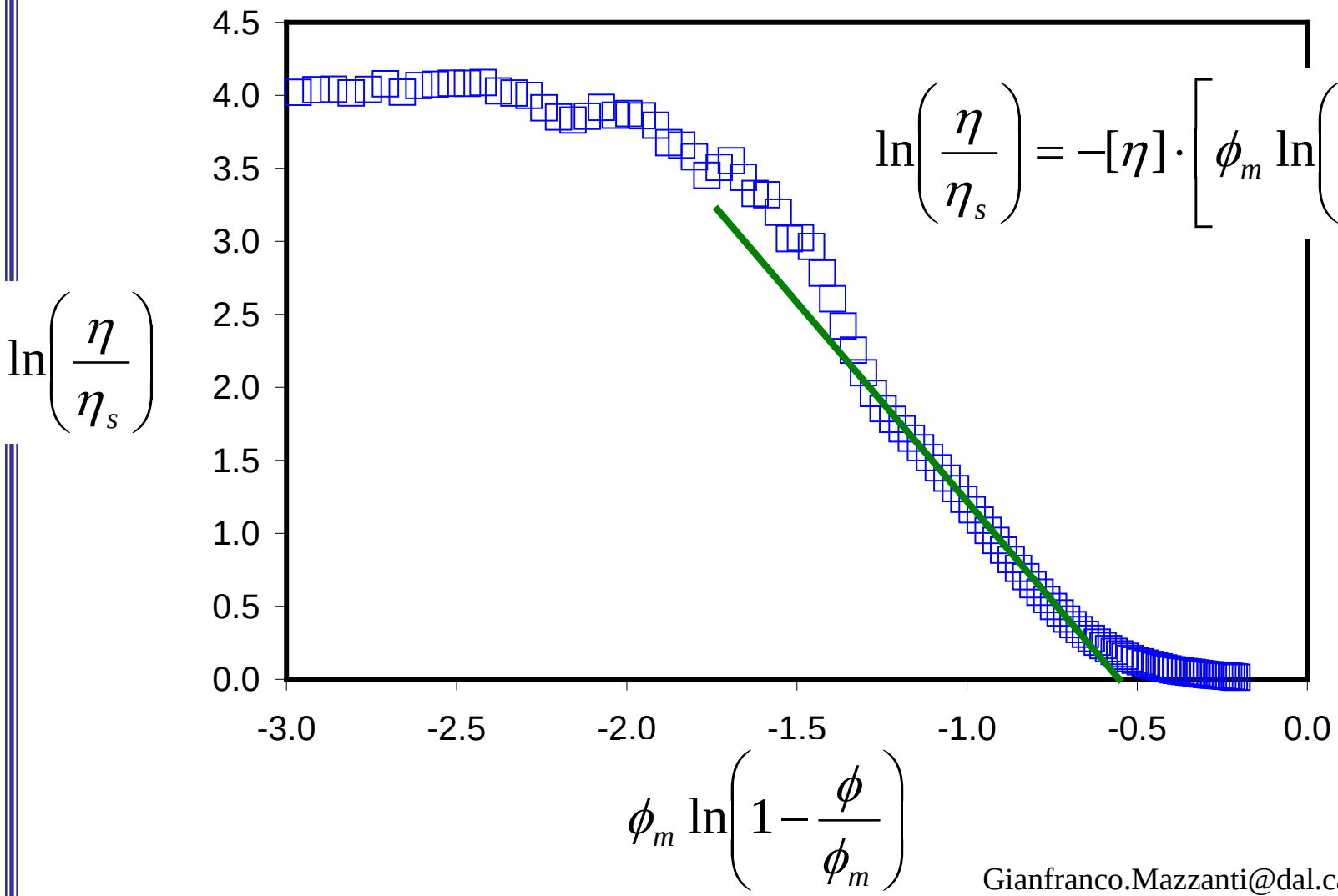
# e.g. Krieger-Dougherty Equation




$$\ln\left(\frac{\eta}{\eta_s}\right) = -[\eta]\cdot\left[\phi_m \ln\left(1-\frac{\phi}{\phi_m}\right)\right]$$

# Other techniques are leading to knowledge on

- Determination of interplatelet forces: Hamacker constant and characteristic distances as function of nanoplatelet surface
- Relationships between modes of aggregation and final structural characteristics

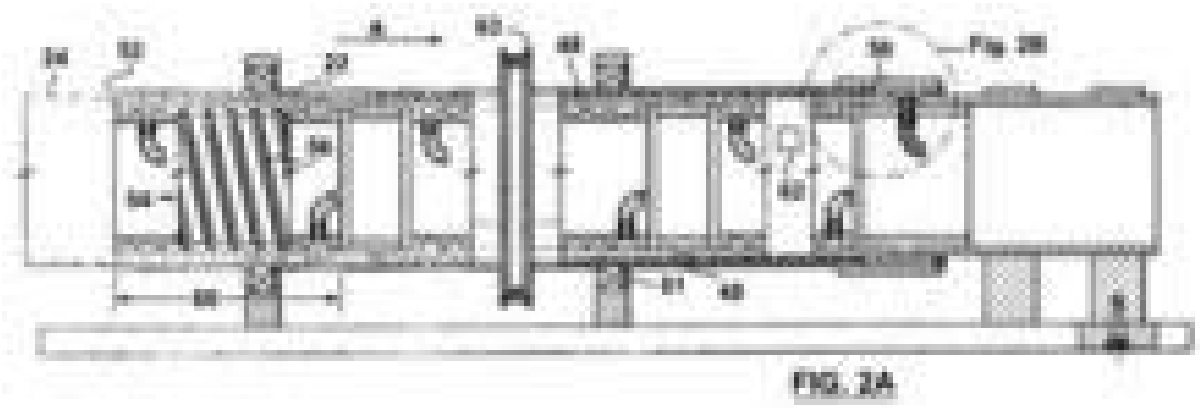
# Summary of knowledge

- Functions of composition and temperature-shear profile to estimate:
  - Characteristic lamellar size, nanoplatelet sizes and orientation related to particle segregation
  - **Accurate** amounts of solids formed under different growth regimes
  - Interplatelet interactions and aggregation
  - Rheological characteristics of the nanoplatelet suspensions during processing
  - Rheological, thermal and diffusional characteristics of the finished material

# Example of application:

**PATENT (WO/2008/119169)**

**Apparatus and method for solidifying a material under continuous laminar shear to form an oriented film (Maleky, Mazzanti, Marangoni, Idziak)**



# Strategies used to improve the stability of L-5-methyltetrahydrofolate

**Yazheng Amy Liu**

Dec 10, 2010

Food Nutrition and Health Program
University of British Columbia, Vancouver, Canada

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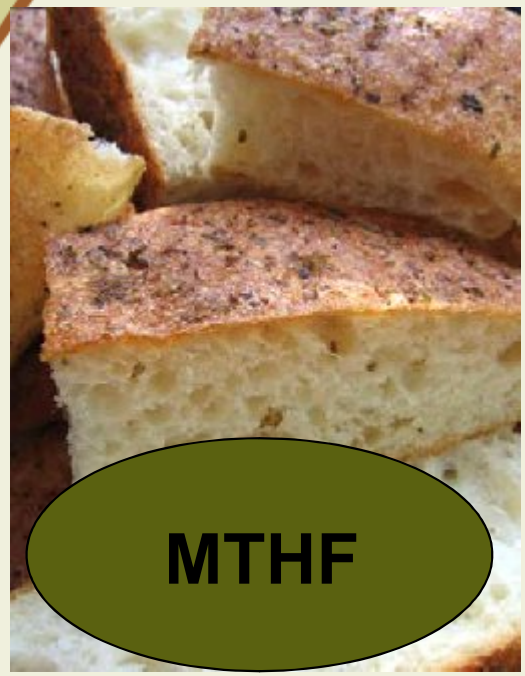
# Folate fortification




**Mandatory folate fortification of enriched grain in US & Canada since 1998 ➡ Reduce NTD**

Folic acid (FA)
- Synthetic

✓ Reduced NTD
- Masking B12 deficiency
- Increase cancer risk

## L-5-methyltetrahydrofolate (MTHF)

- Natural, reduced

✓ not mask B12 deficiency

✓ Safe fortificant
- Unstable

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# Outline

- Stability study of MTHF
- Interactions between antioxidants and MTHF
- Stabilization of MTHF using microencapsulation
- Summary and Discussion

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# Stability study of free MTHF

- **Model system**

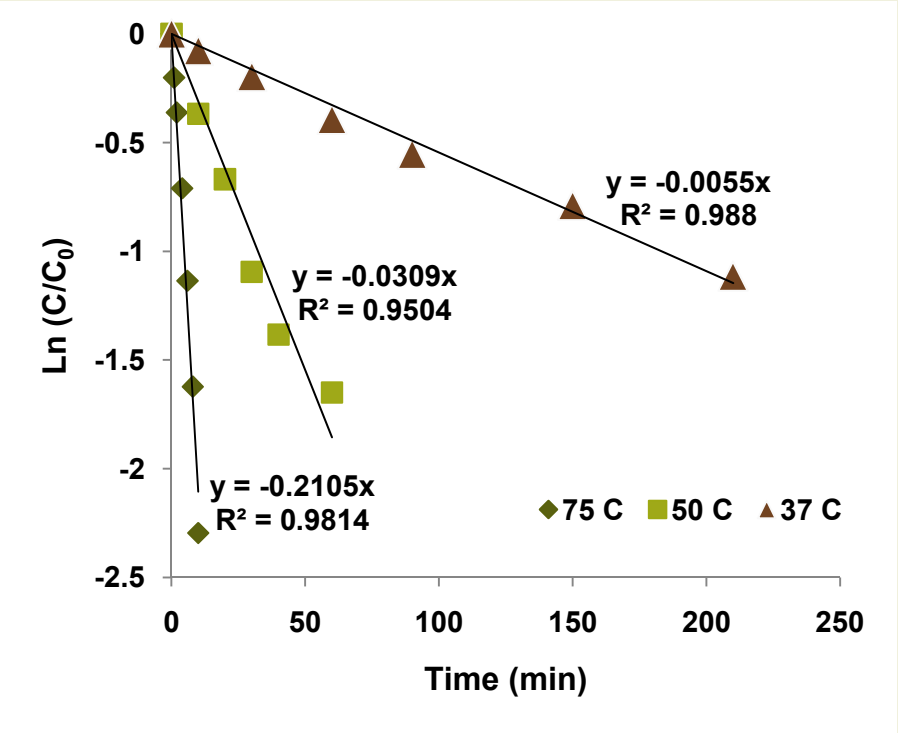



**Fig 1.** Thermal degradation kinetics of MTHF (50µg/ml, pH 6.8, limited $O_2$)

- **Food system**

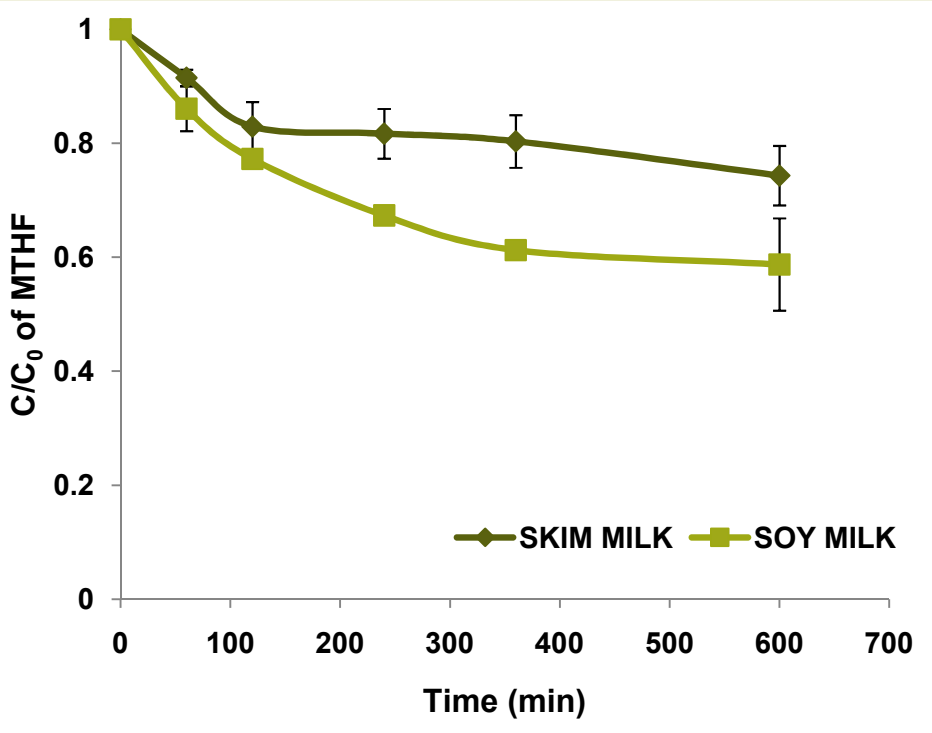



**Fig 2.** Degradation of MTHF in skim milk and soy milk (50ºC)

# Interactions between antioxidants and MTHF

- **Improvement of thermal stability**

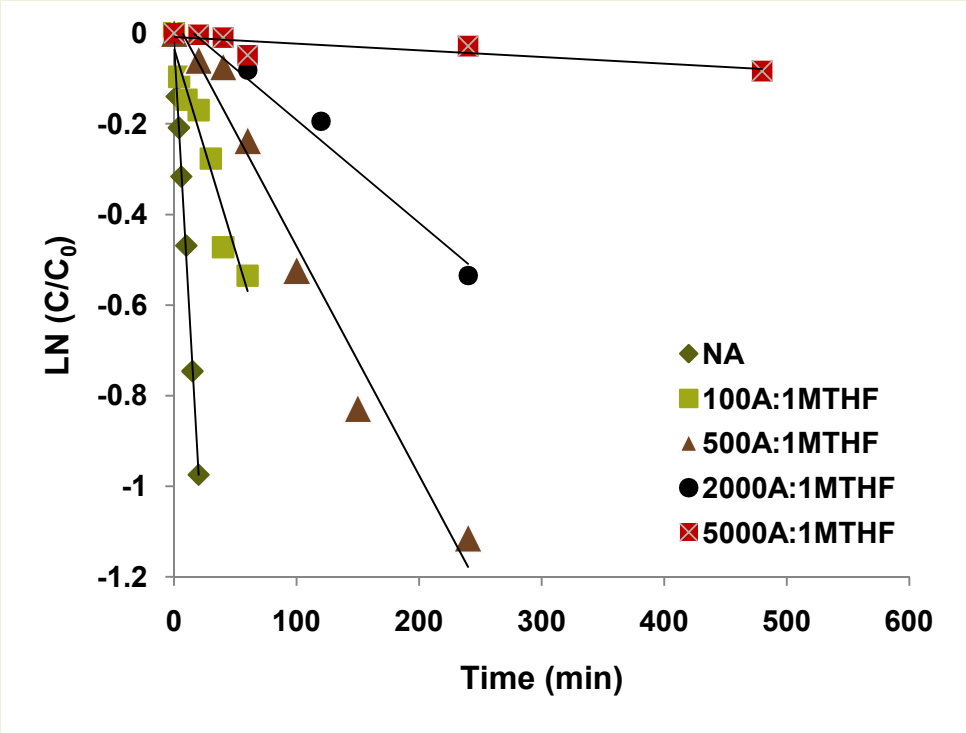



**Fig 3.** Thermal degradation of MTHF containing different molar ratios of sodium ascorbate (A) (50ºC, pH 6.8)

- **Return study of MTHF by sodium ascorbate (NaAc)**

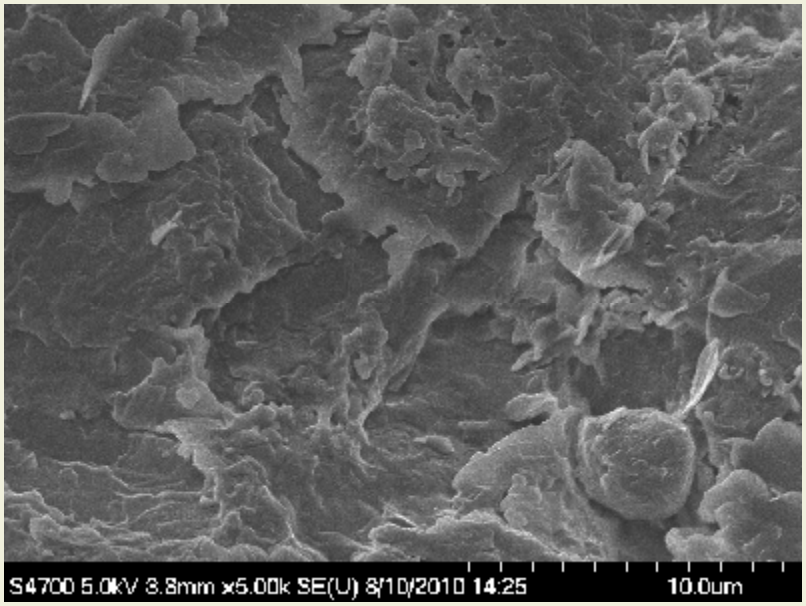
**Tab 1.** Regeneration of MTHF by NaAc

| | 60 min | 150 min |
|---|---|---|
| NA | 0.68±0.09 | 0.41±0.06 |
| 0.1% NaAc | 0.80±0.02 | 0.55±0.06 |
| 1% NaAc | 0.98±0.03 | 0.87±0.04 |
| 2% NaAc+MCE | 0.99±0.03 | 0.92±0.00 |

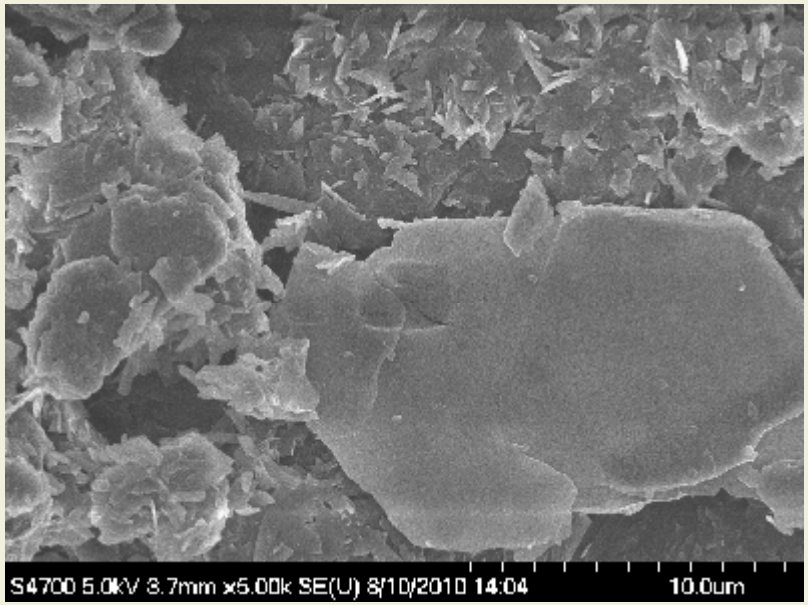
* MTHF was heated at 50ºC for 60min and 150min, then antioxidants were added. Data are expressed as $C/C_0$

# Stabilization of MTHF using microencapsulation

- **Electronmicroscope pictures**



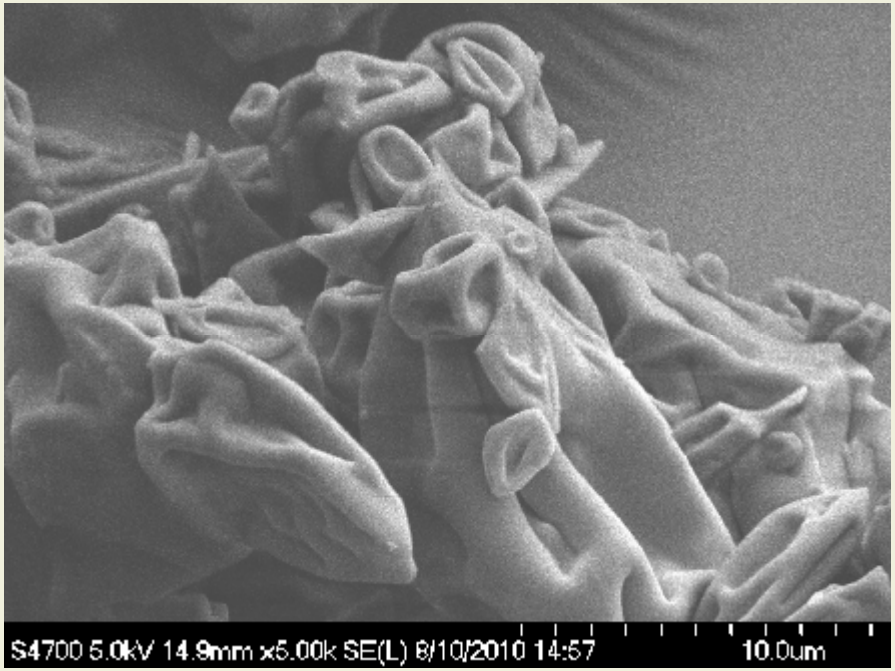

**Free MTHF**



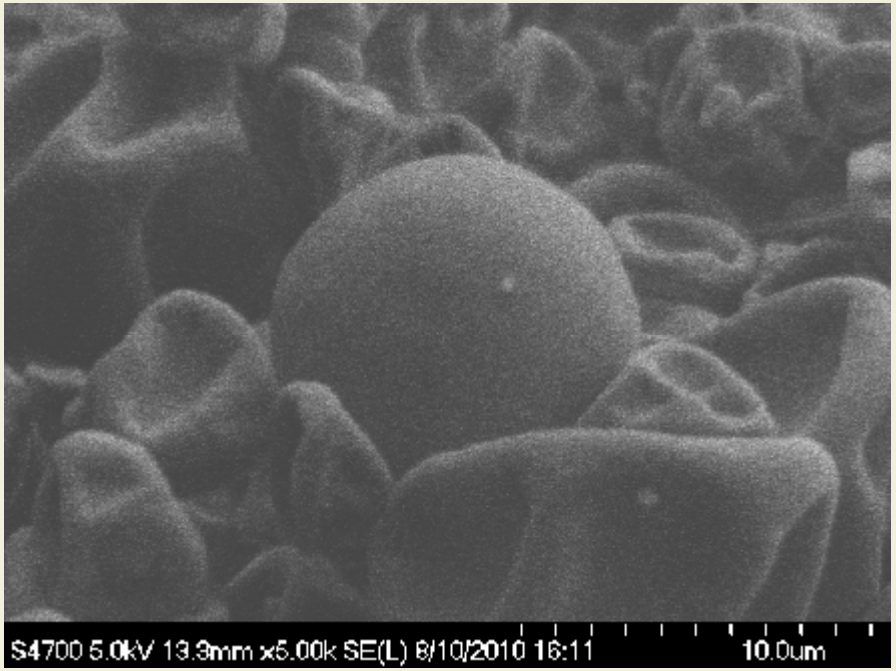

**Stearate coating**

# Stabilization of MTHF using microencapsulation

- **Electromicroscope pictures**




**PS coating**




**Skim milk MTHF-spray dry**

# Stabilization of MTHF using microencapsulation

- **Loss of MTHF during microencapsulation process**
  - Pectin-alginate: > 80%
  - Stearate coating: ~44%
  - PS coating: ~ 30%; PS(MTHF+AA): NO

- **Encapsulated MTHF is stable at RT during storage.**

  - all coatings: >95% after 3 months

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# Stearate MTHF

- **Model system**

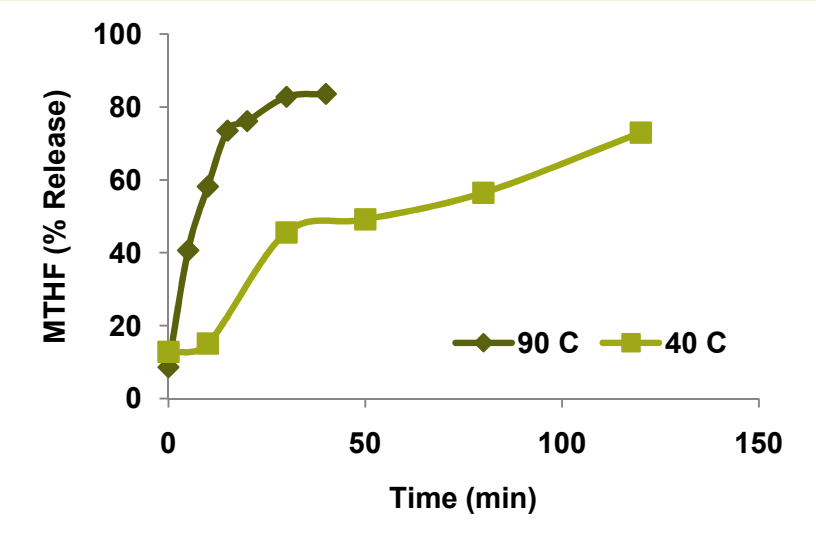


**Fig 4.** Release of MTHF from stearate coating to the buffer system

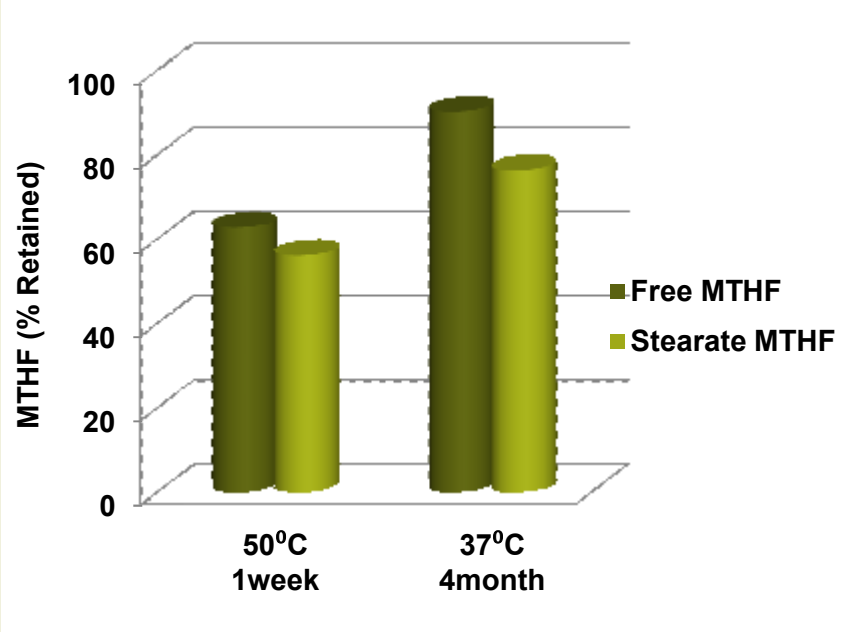


**Fig 5.** Stability of free and encap MTHF (dry form)

# Stearate MTHF

- **Food system**

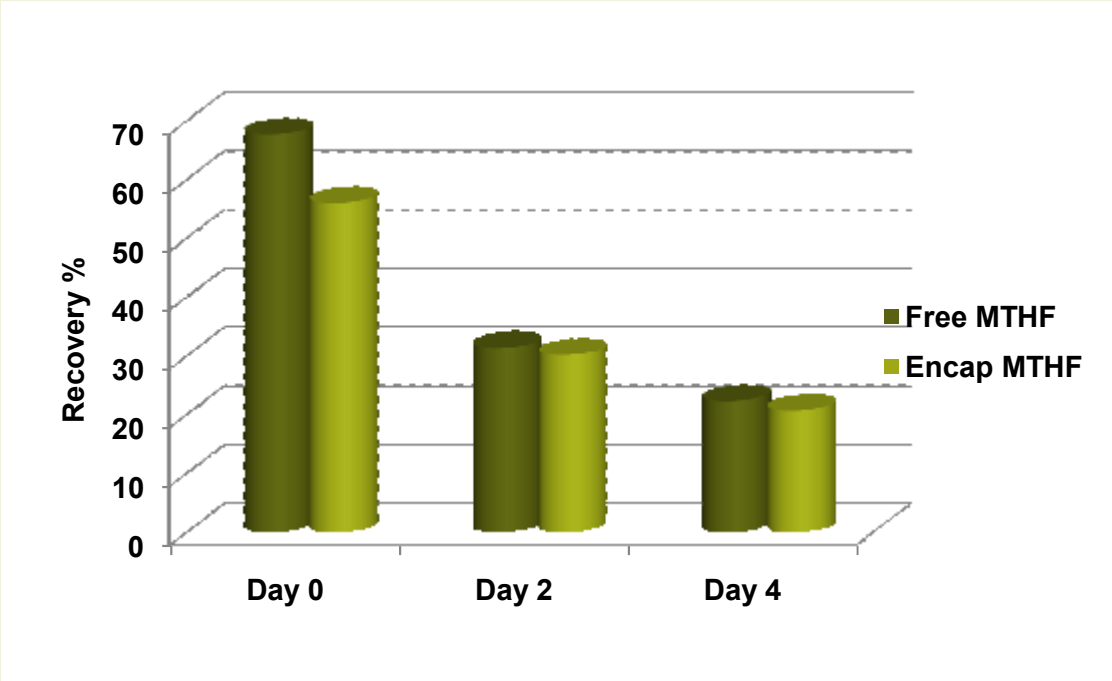


**Fig 6.** Recovery of MTHF in bread (after baking and during storage at RT)

# PS MTHF & PS (MTHF+AA)

- **Food system**

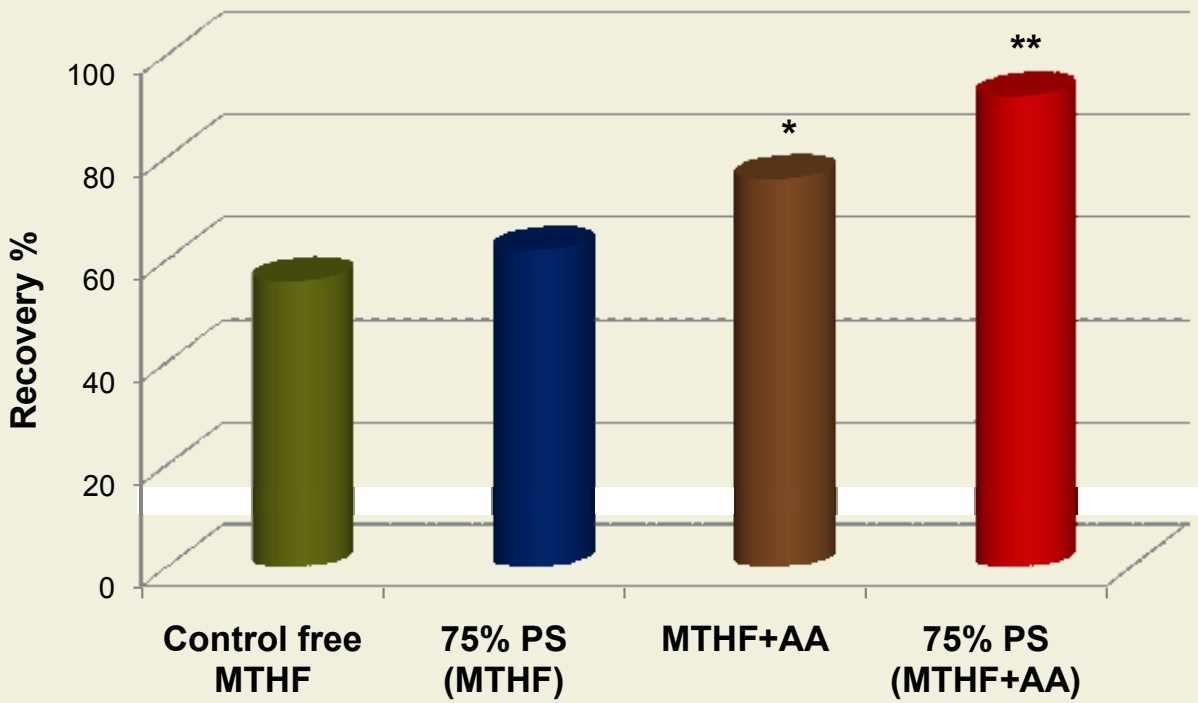


**Fig 7.** Recovery of MTHF in bread after baking

# Summary & Discussions

- MTHF is labile to heat, first order kinetics under limited $O_2$
- Antioxidants can stabilize MTHF and return certain degradation products back to the initial form
- Lost of MTHF during bread baking and further storage; relatively stable in milk and soy milk may be due to the antioxidant property
- The pectin-alginate matrix was not effective coating material
- Stearate coating has limited potential for protecting thermally unstable MTHF

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# Summary & Discussions

- The PS coating showed greater coating potential
- The combination of micro/nano-encapsulation with stabilizer resulted in the greatest potential for stabilizing MTHF

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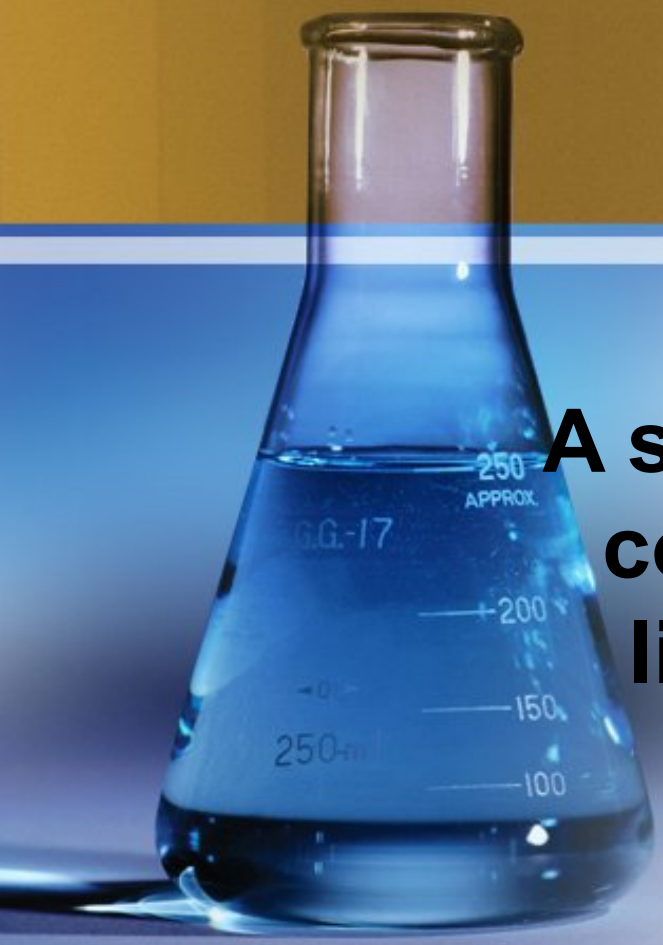
WHISTLER CENTER
for Carbohydrate Research



# A soluble nanoscale self-assembling complex from starch, protein, and lipid for healthy nutrient delivery


**Deepak Bhopatkar**
**Osvaldo H. Campanella**
**Bruce R. Hamaker**

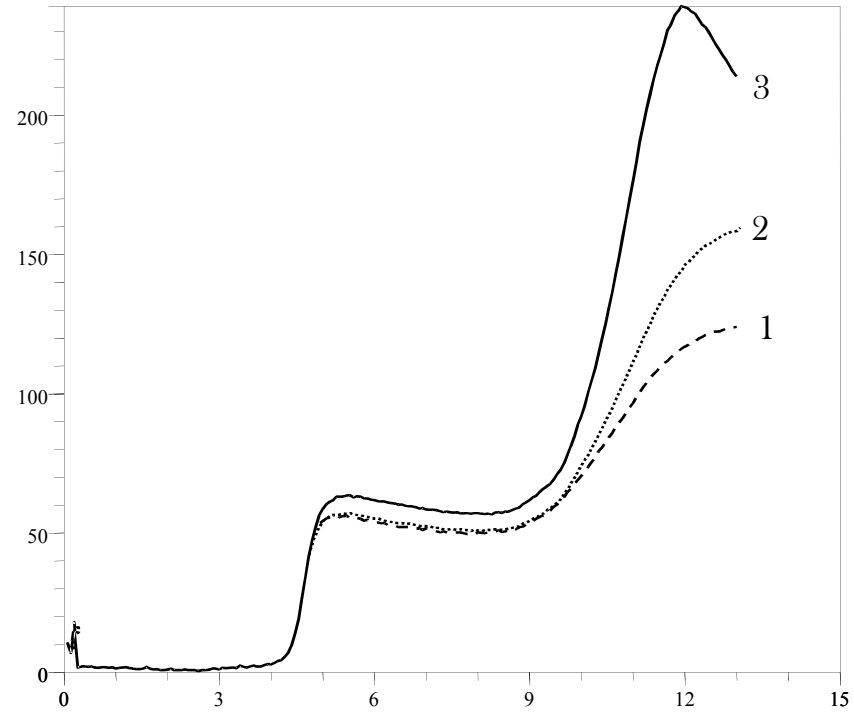
PURDUE UNIVERSITY

# THE 3-COMPONENT COMPLEX

- A self-assembled nanoscale complex formed with common macronutrient

- Complexation achieved **only** when all the components were present in a particular ratio

- Components associate via electrostatic bonding between the well known hydrophobic interaction-driven amylose-FFA complex and certain disulfide-linked soluble proteins.

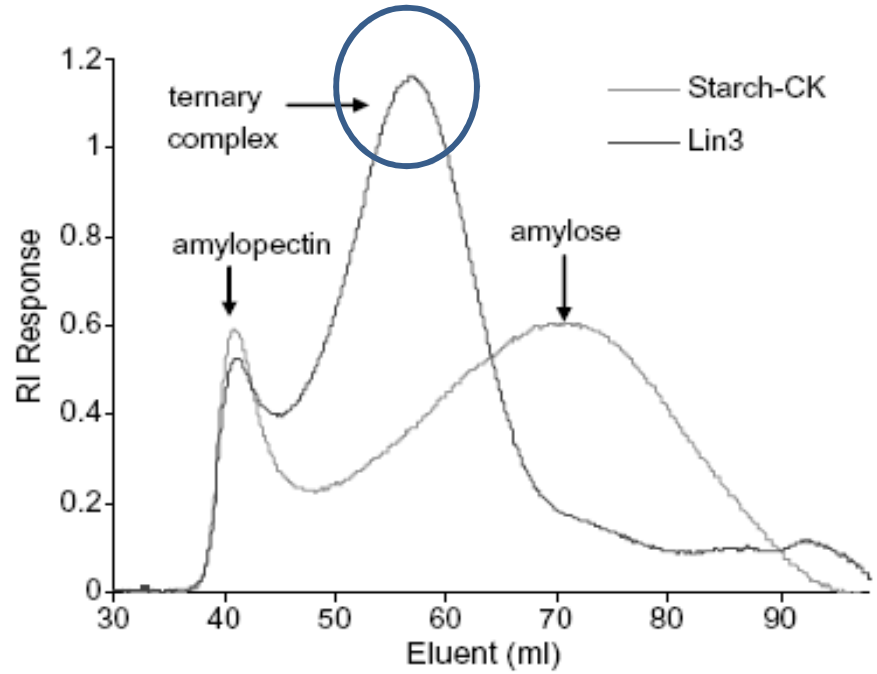


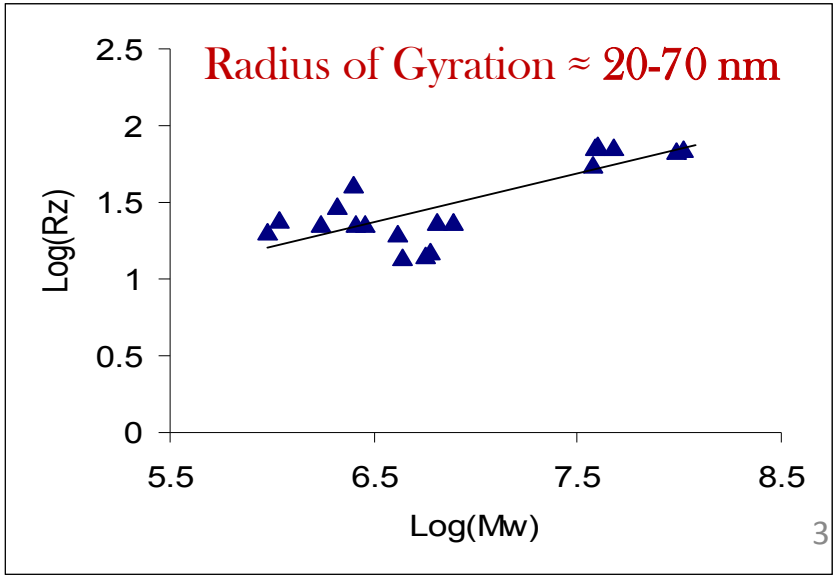

1) Starch 2) Starch + FFA** 3) Starch + Protein + FFA **

* RVA: Rapid Visco Analyzer
** FFA : Free Fatty Acid

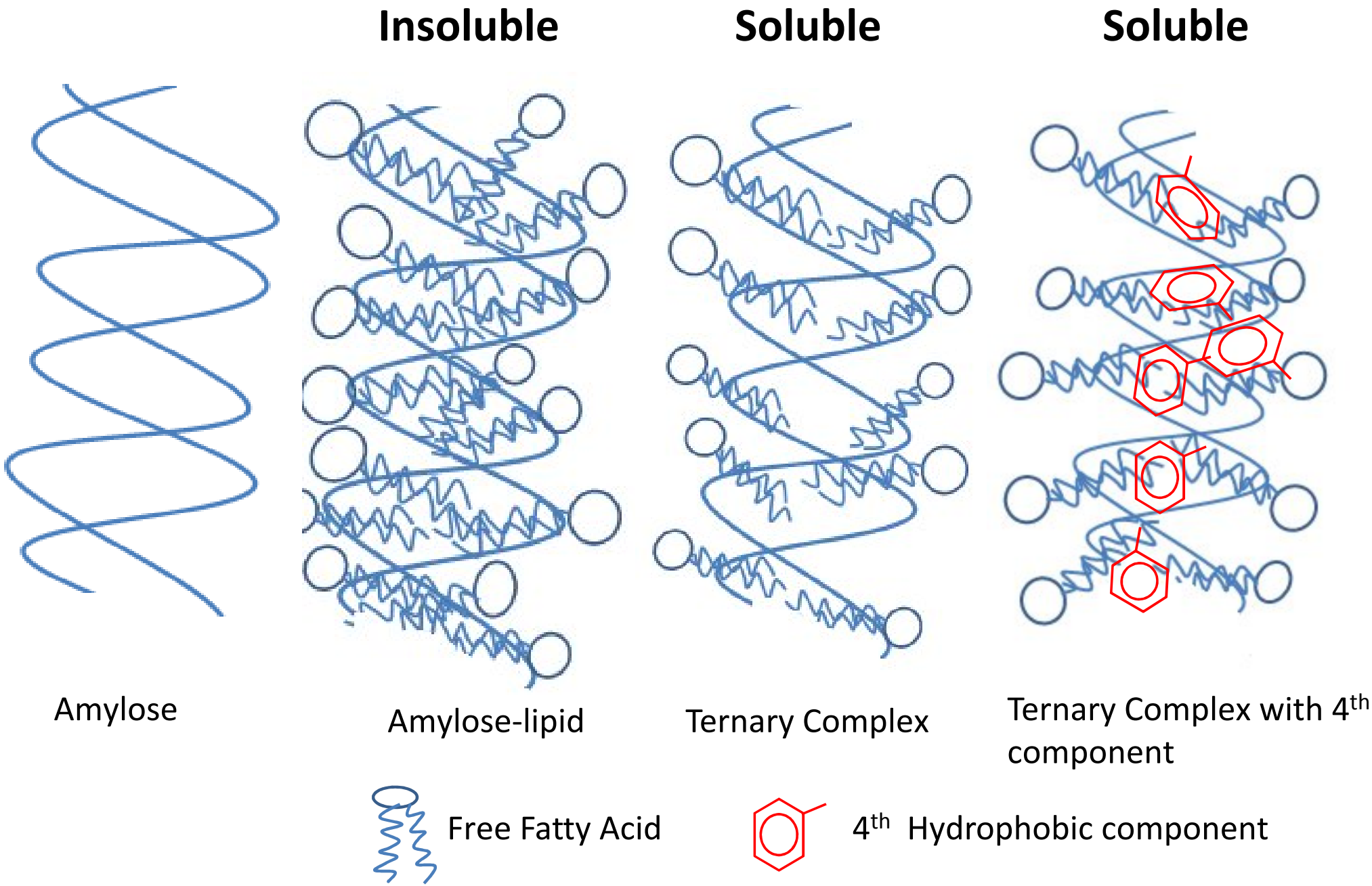
# IDENTIFICATION OF 3-COMPONENT COMPLEX

- The nanocomplex can be separated by size exclusion chromatography (SEC)
- This "soft" nanoparticle has a size about 50-150 nm
- Complexation is achieved in a dilute system
- DSC, X-ray diffraction confirmed the complexation

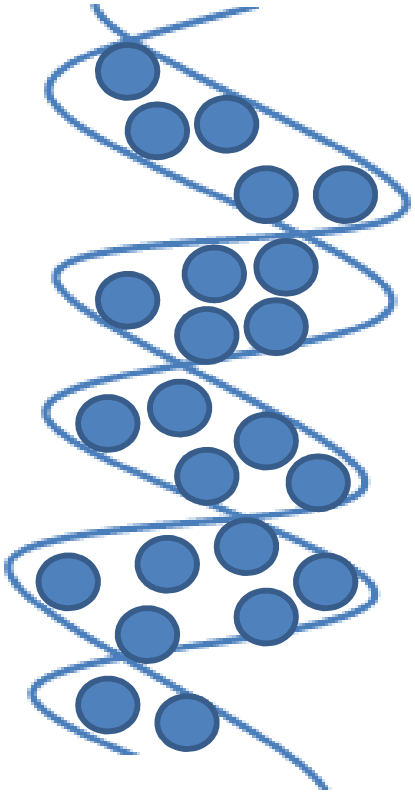


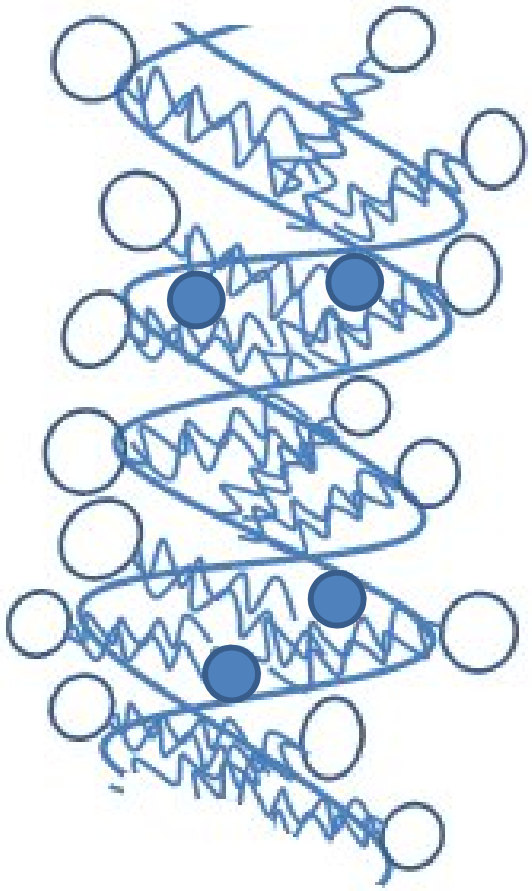
# Novel Aspects of Ternary Complex



# SCHEMATIC REPRESENTATION OF IODINE INCLUSION – POTENTIOMETRIC CONCEPT



Amylose

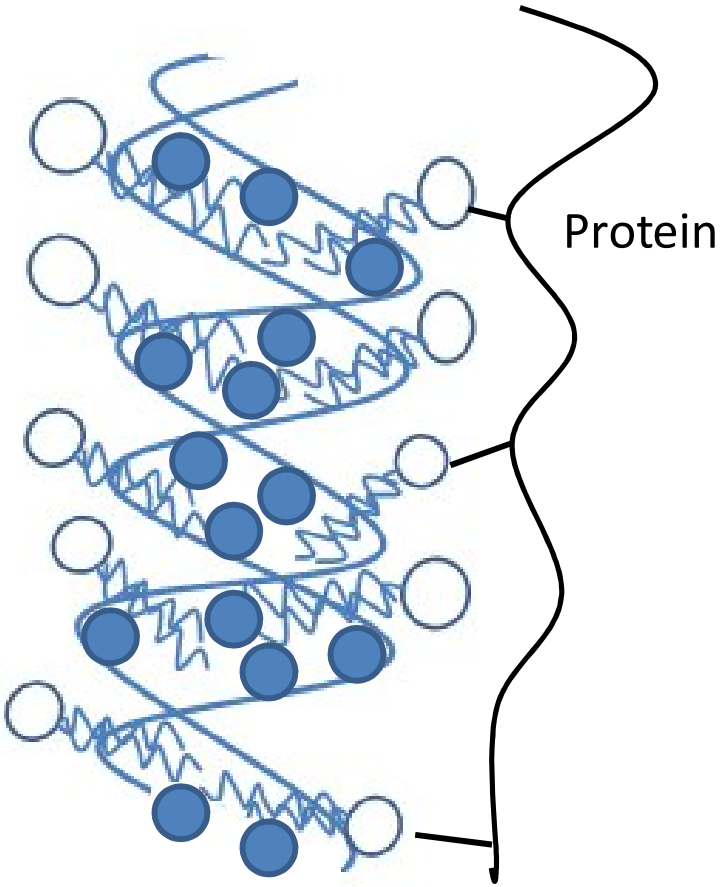


Amylose-lipid

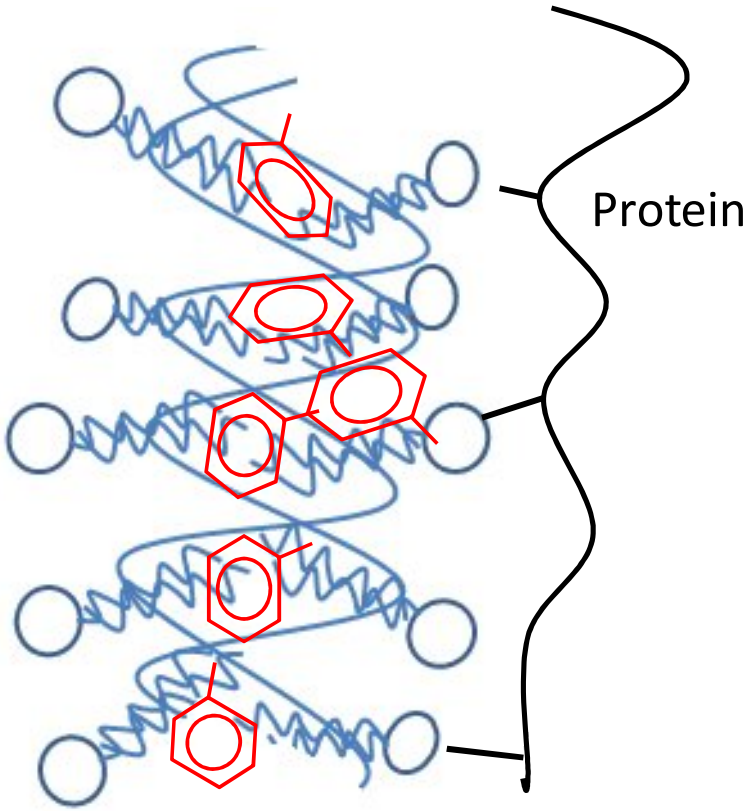
Free Iodine ($I_3$)

Free Fatty Acid

# SCHEMATIC REPRESENTATION OF IODINE AND 4th COMPONENT INCLUSION




Ternary Complex



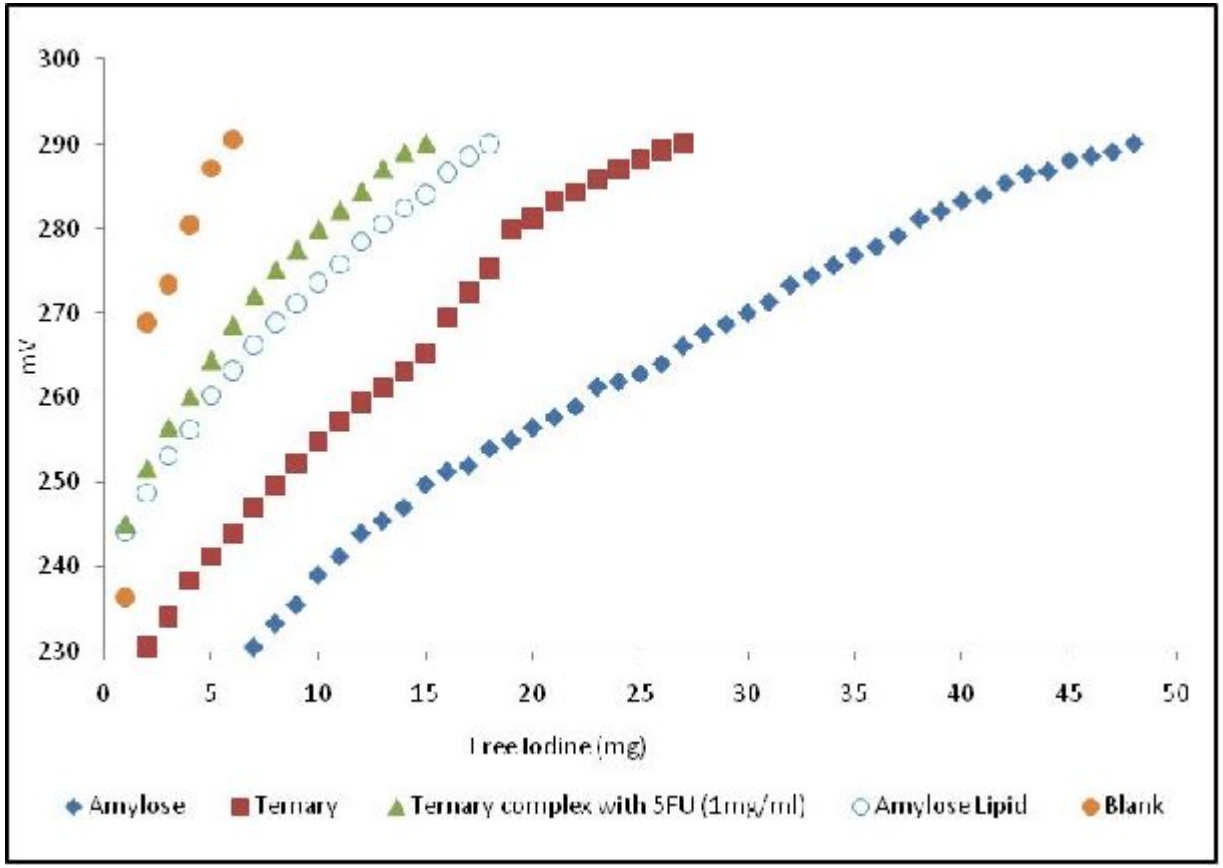

component

Free Iodine ($I_3$)

Free Fatty Acid

4th Hydrophobic component

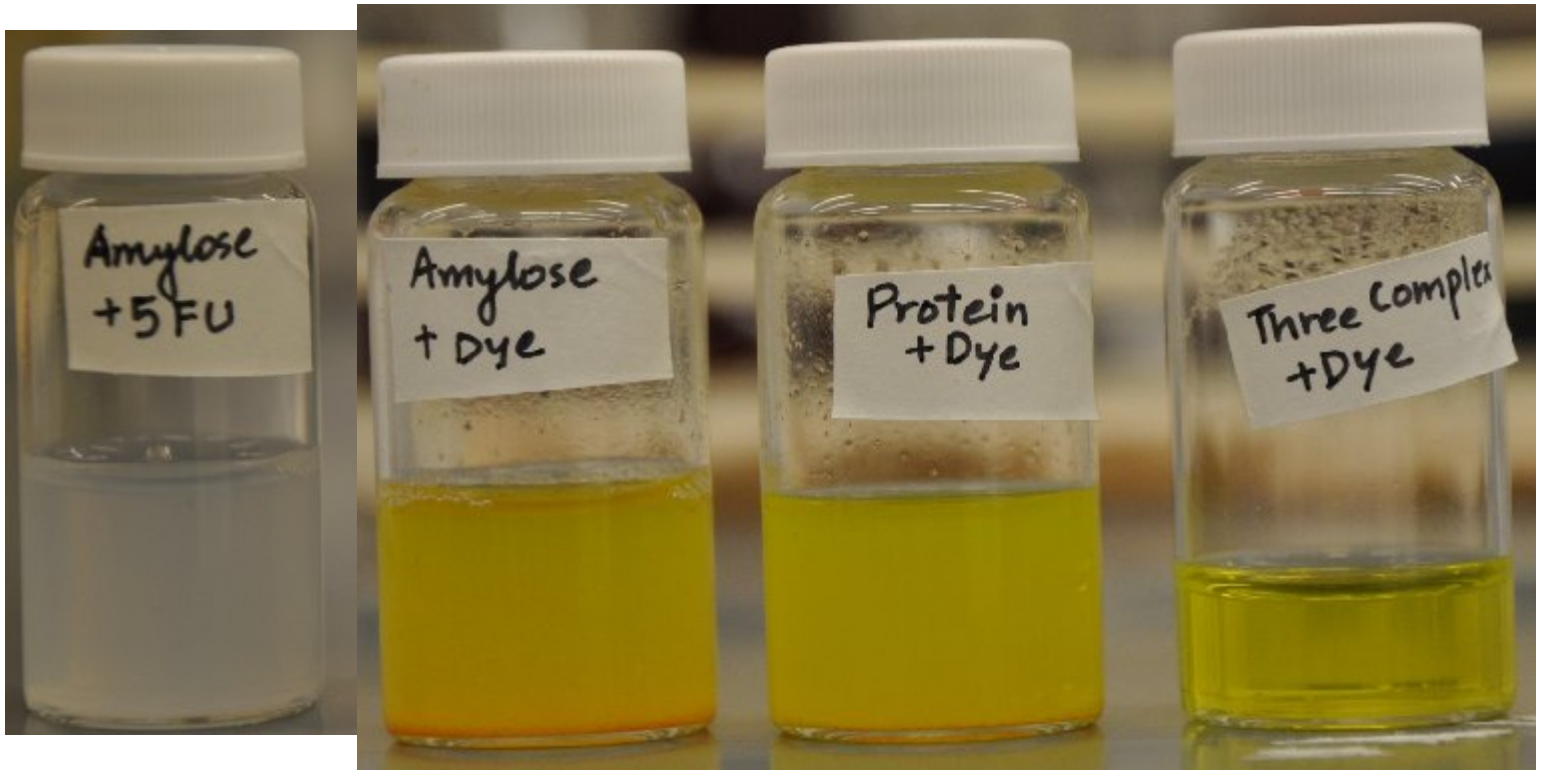
# POTENTIOMETRIC EVIDENCE



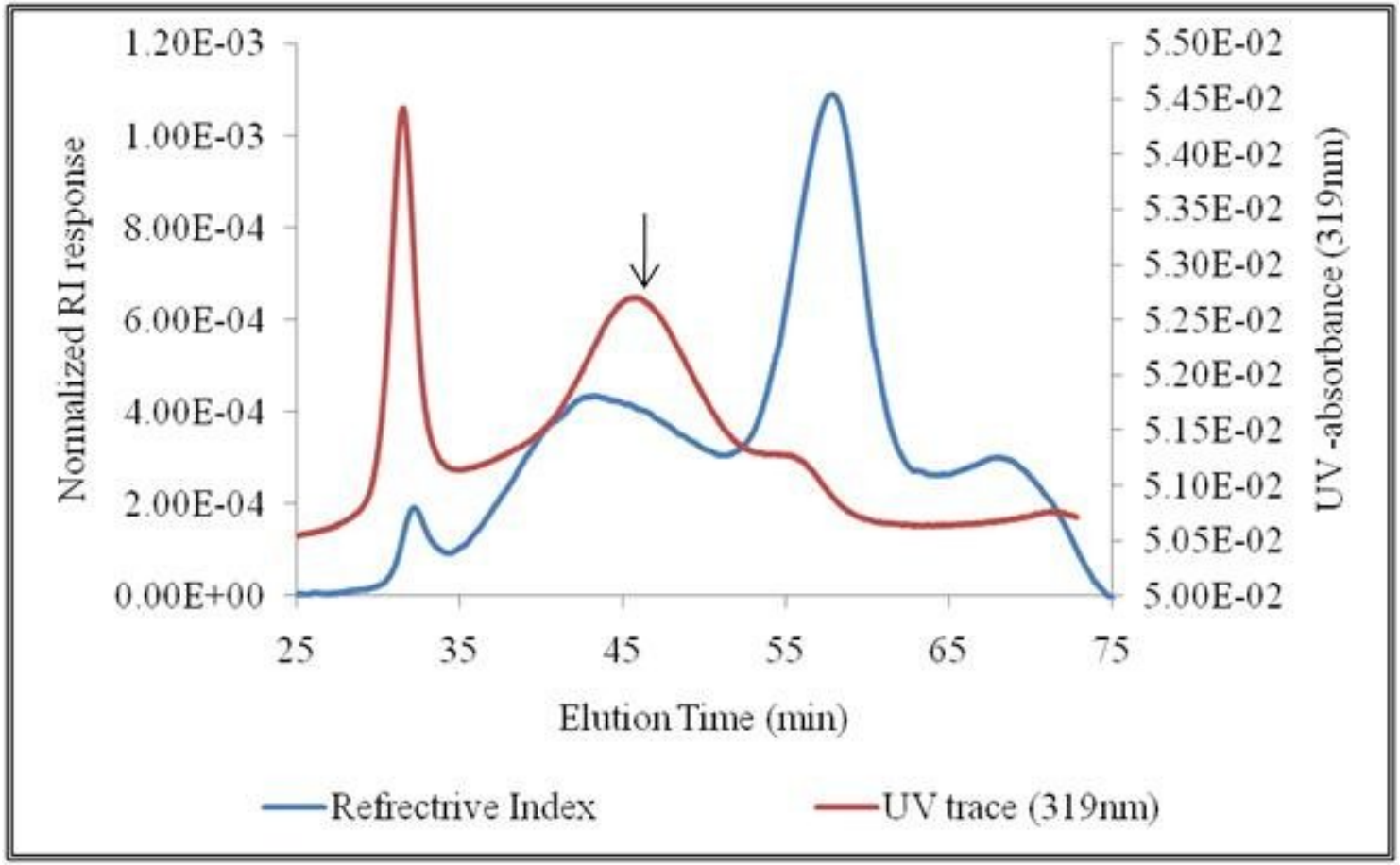
# INCLUSION OF MODEL HYDROPHOBIC COMPOUNDS

## Model Compounds

- α Napthol (antimicrobial compound)
- 5-Flurouracil (chemotheraputic drug)
- Fluorescein (dye)



# CHROMATOGRAPHIC EVIDENCE




- Solubilization of α-napthol through inclusion with ternary nanocomplex indicated by UV peak associated with the nanocomplex in HPSEC profile

# CONCLUSIONS

- Ternary complex can solublize small hydrophobic compounds (antimicrobials, drugs, dyes)

- Possible uses:
  - Enhanced and/or targeted delivery of nutraceuticals, antimicrobials and drugs
  - Controlled release of flavors
  - Application in masking the flavor for improved sensory quality of final product