USDA-NRI Progress Report (12/10/2010)

Nanostructure and Nanoscale Interactions of Protein/Polysaccharide Complexes

Project Director: Qingrong Huang

Rutgers University

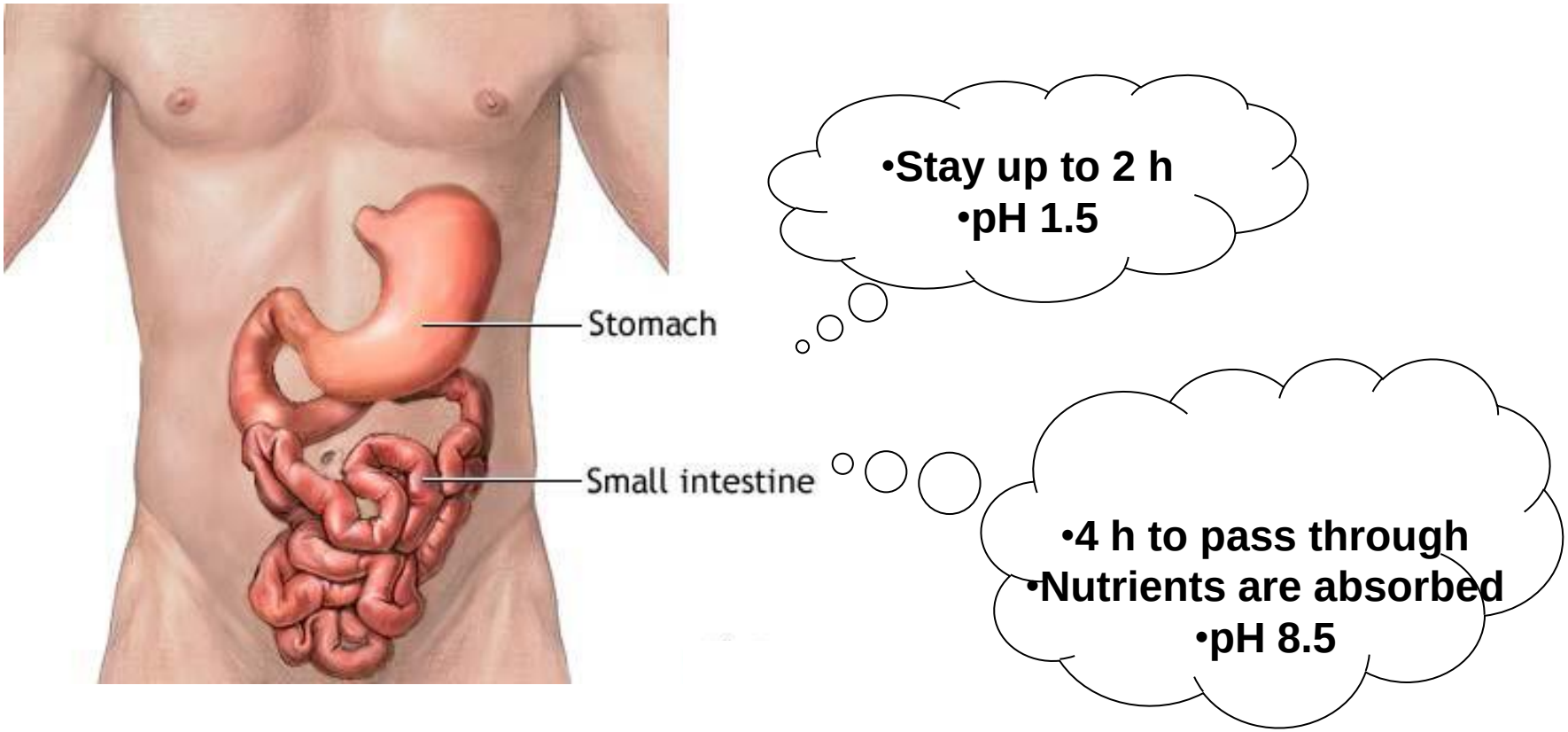
Functional Foods

- Global functional food market will reach $90.5 billion in 2013;
- "foods and food components that provide a health Benefit beyond basic nutrition" – IFT expert report
- Usually accompanied by health claims for marketing purposes.

HIGH ORAC VALUE

(ORAC: *oxygen radical absorbance capacity*)

Absorption of Nutraceuticals in Gastrointestinal Track: (1) Micro/nanoencapsulation of nutraceuticals and micronutritions; (2) Controlled release at small intestine.



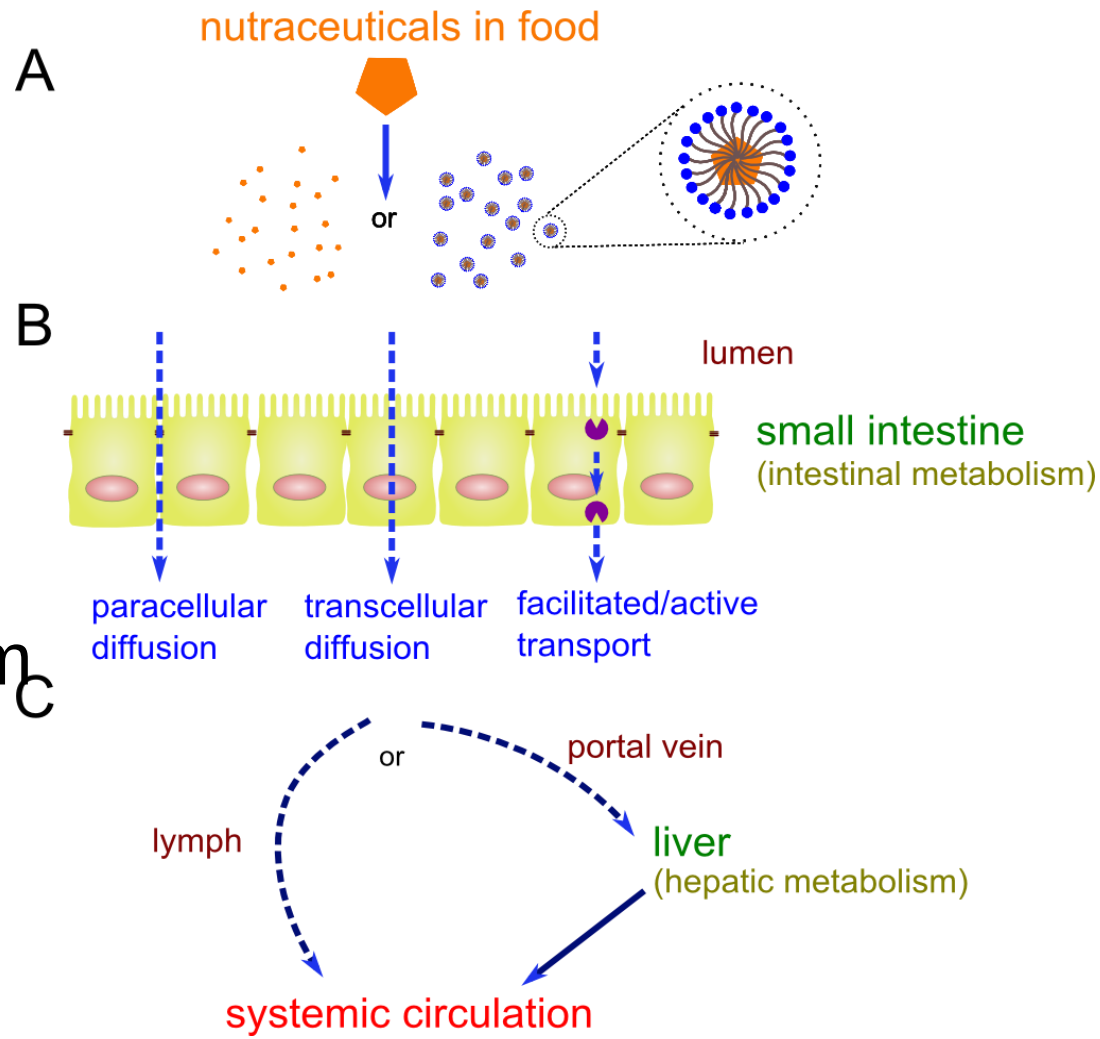
Main objective: Develop novel state-of-the-art technologies to characterize nanostructure and nanoscale interactions of protein/polysaccharide coacervates, and use the knowledge obtained to rationally design novel food delivery systems with controlled-release characteristics.

Biological Fate of Nutraceuticals

1. Solubilization

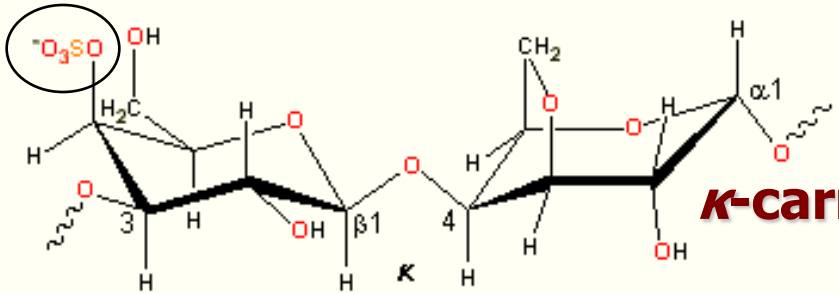
2. Absorption

3. Escape metabolism



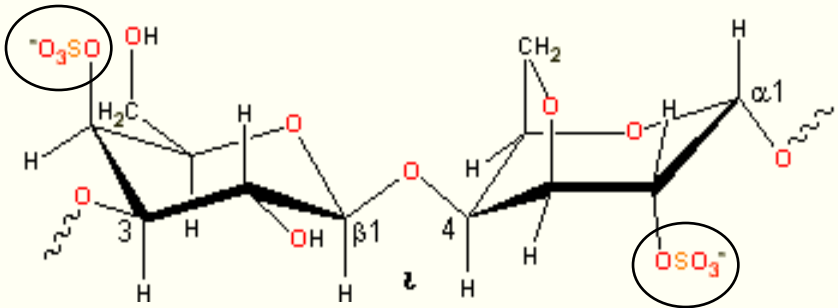
Strong Polyelectrolytes

- Carrageenans
  - Naturally sulfated polymer
  - Various kinds, difference in linear charge density(ξ)



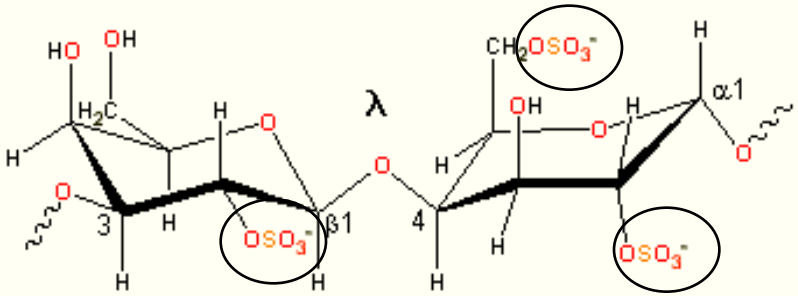
κ-carrageenan (ξ=0.92)

-(1→3)-b-D-galactopyranose-4-sulfate-(1→4)-3,6-anhydro-a-D-galactopyranose-(1→3)-



ι-carrageenan (ξ=1.53)

-(1→3)-b-D-galactopyranose-4-sulfate-(1→4)
-3,6-anhydro-a-D-galactopyranose-2-sulfate-(1→3)-

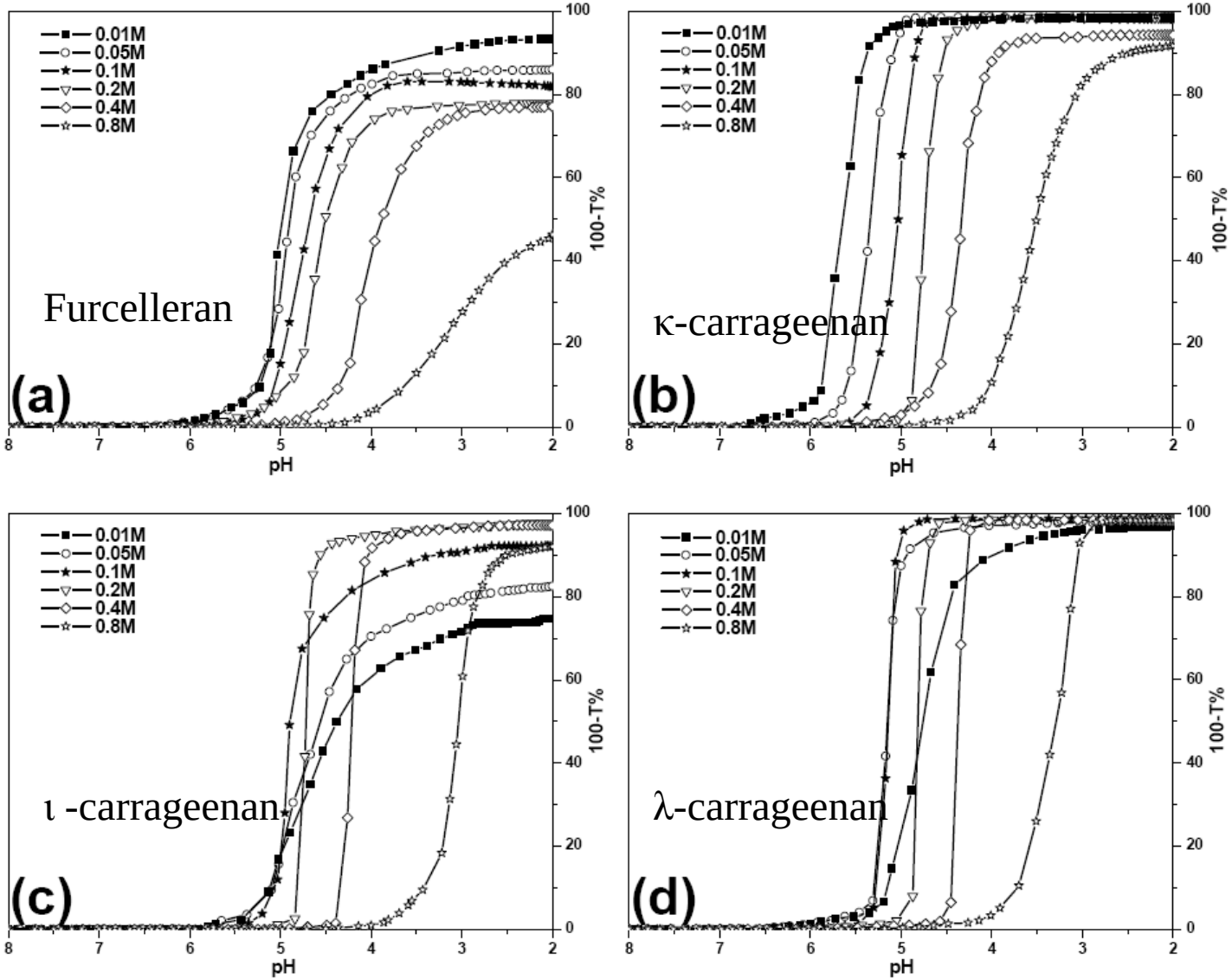


λ-carrageenan(ξ=2.07)

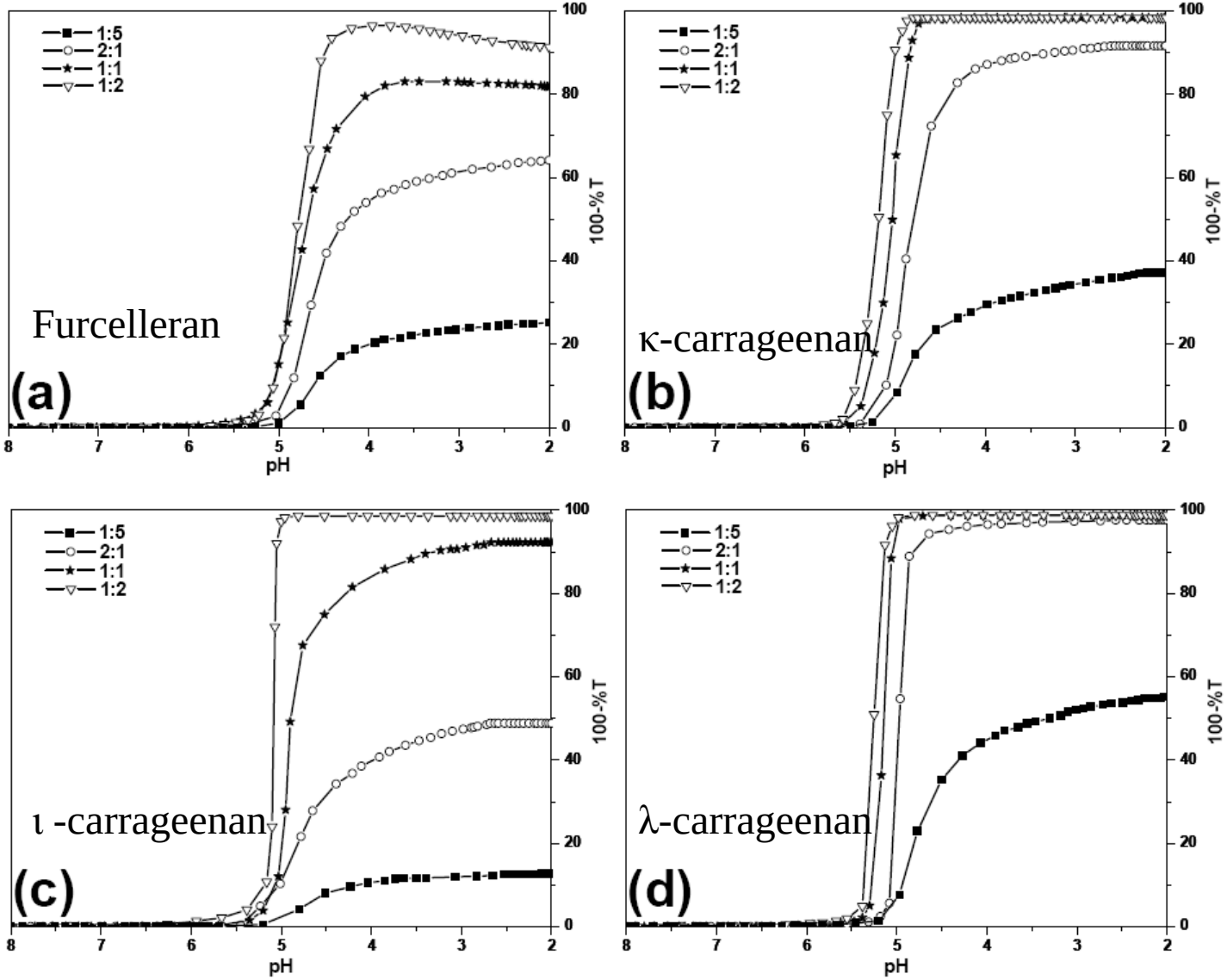
-(1→3)-b-D-galactopyranose-2-sulfate-(1→4)
-a-D-galactopyranose-2,6-disulfate-(1→3))-

(de Ruiter, G. A. *et al*. **1997,** *Trends in Food Science & Technology*)

Effects of Salt Concentrations

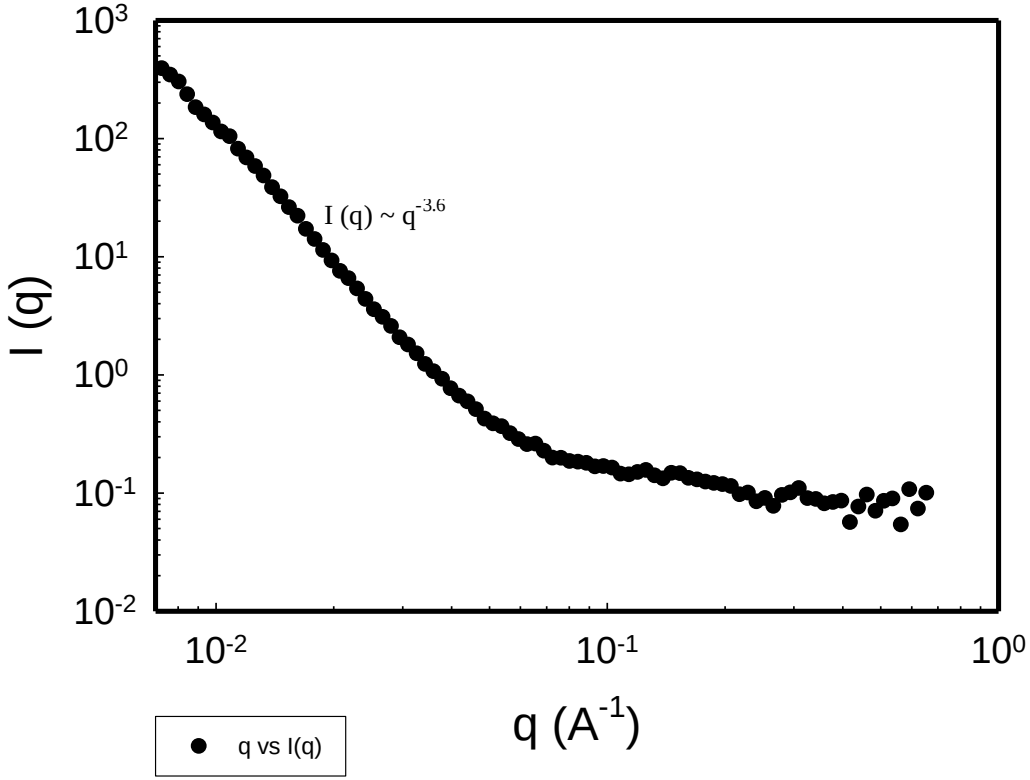


Effects of Protein/Polymer Ratios

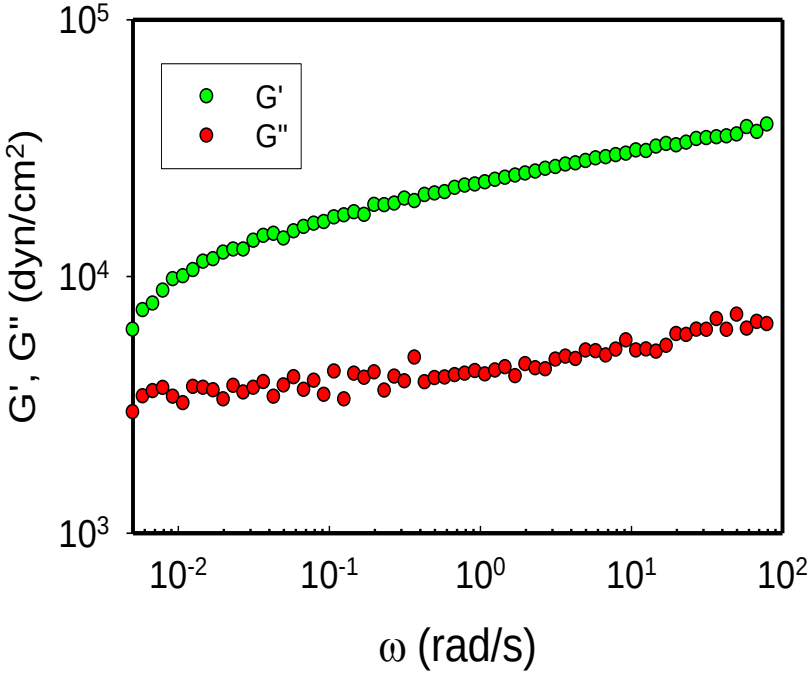


Structure and Rheological Properties

κ-Carrageenan/b-lg (1:10) in 0.1 M NaCl, T=25 °C, pH=4.5

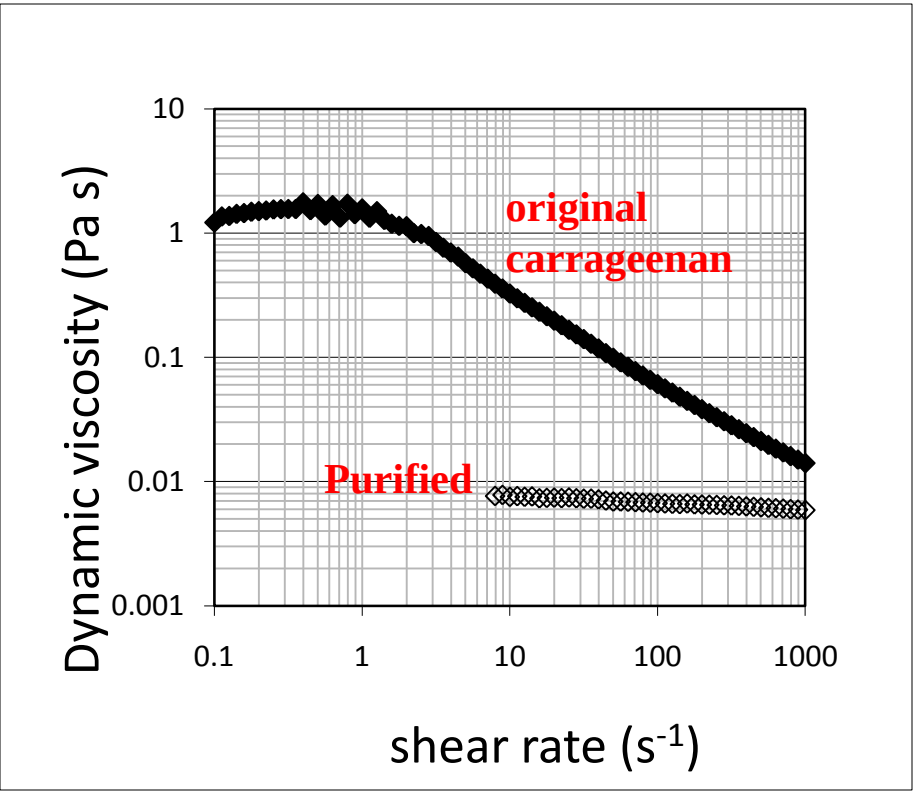


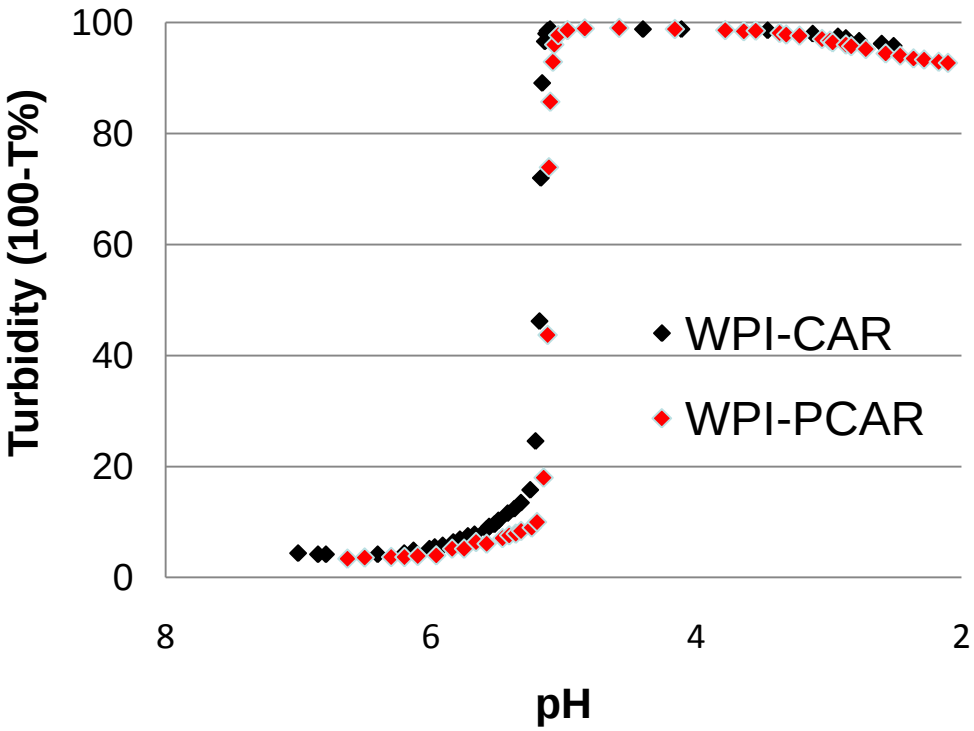
Small-angle neutron scattering



Rheology

Effects of Cationic Types

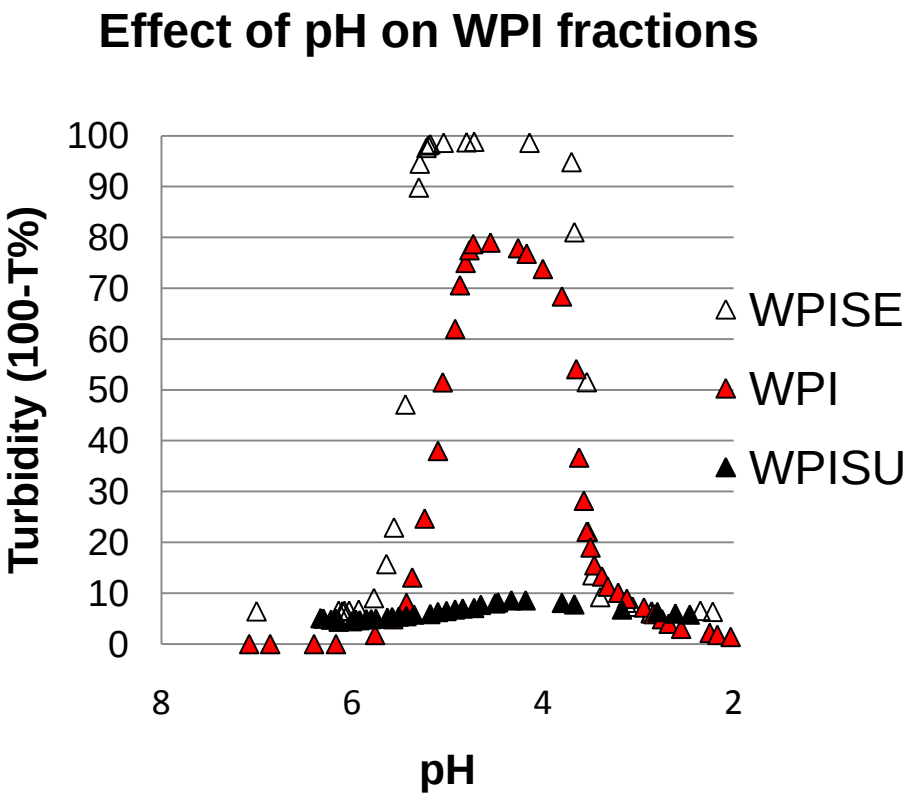




purified ι-carrageenan interactions

Cation type (Na+ vs. Ca2+) affects the rheological properties of the complexes, but has negligible effect on phase diagrams.

Phase Diagrams of WPI Fractions

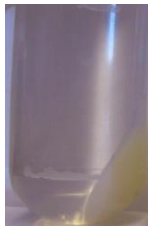


- WP treatment

Dispersion at pH 5

⇩

Centrifugation



WPI

⇩

Filtration

⇩

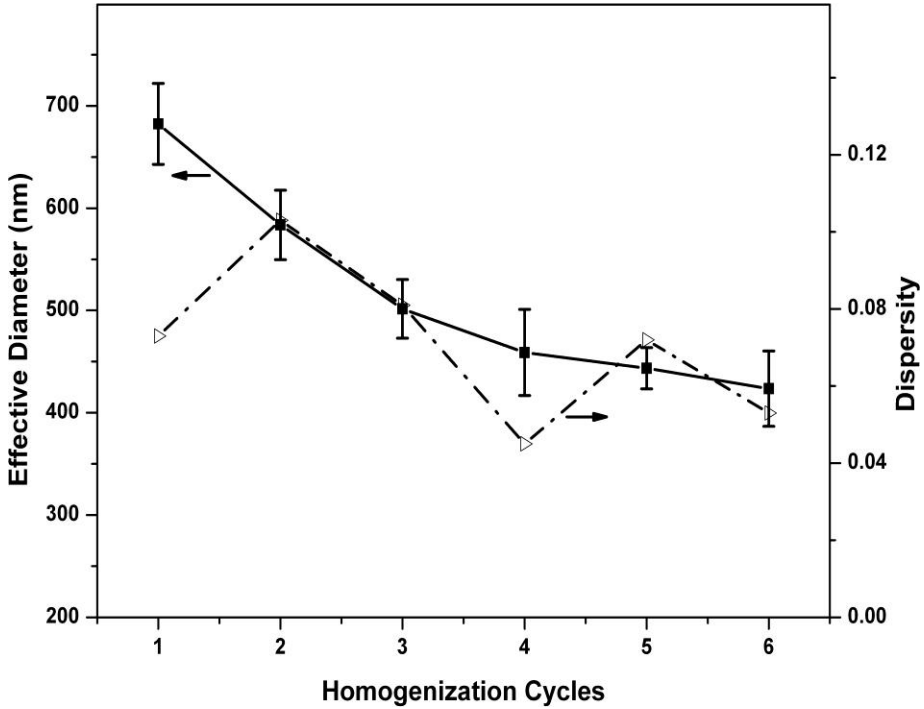
Freeze-drying

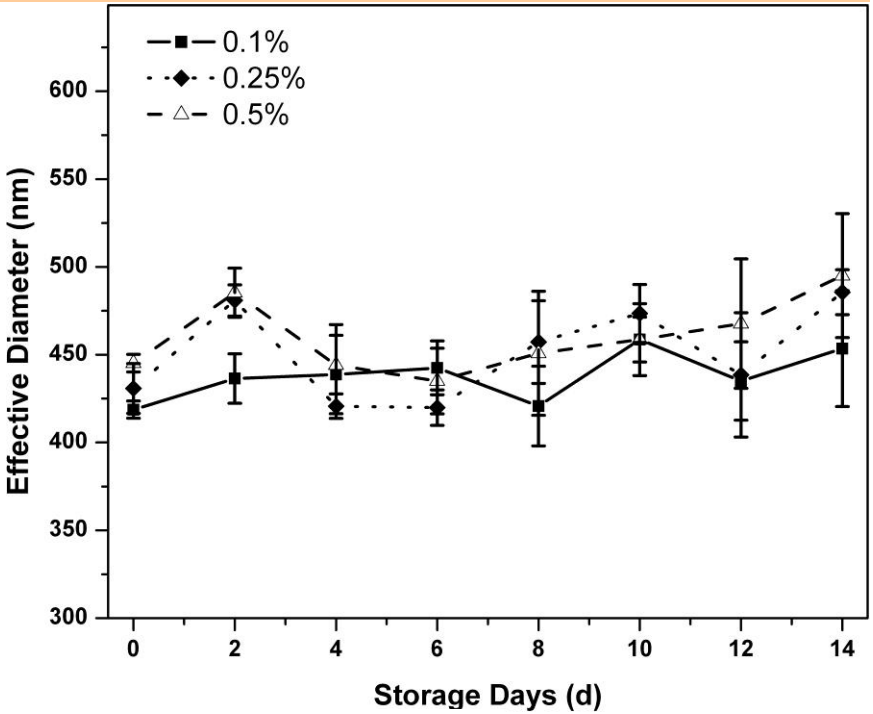
Powder characteristics:

Supernatant : white, powder, highly soluble (WPISU)

Sediment: yellowish, grainy texture, less soluble (WPISE)

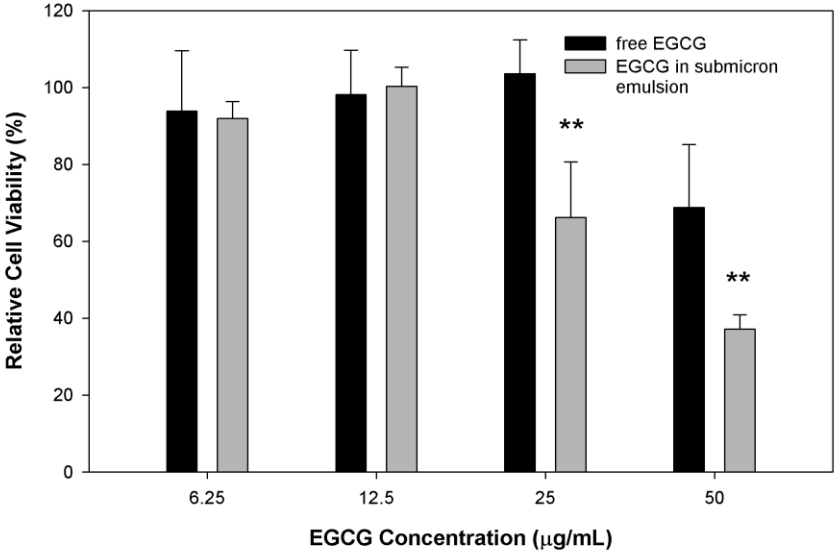
Improve In Vitro Anti-Cancer Activity of EGCG





Emulsion size decreased with the increase of homogenization pressure.

Carrageenan/β-lg stabilized EGCG sub-micrometer O/W emulsion showed improved in vitro Anti-cancer activity aganist Human HepG2 cancer cells.



The emulsion sizes have negligible change during 14 days storage.

EGCG: epigallocatechin-3-gallate

Cationic Biopolymer:Chitosan

Chitosan is a cationic polysaccharide

is a fully or partially N-deacetylated product of naturally abundant chitin

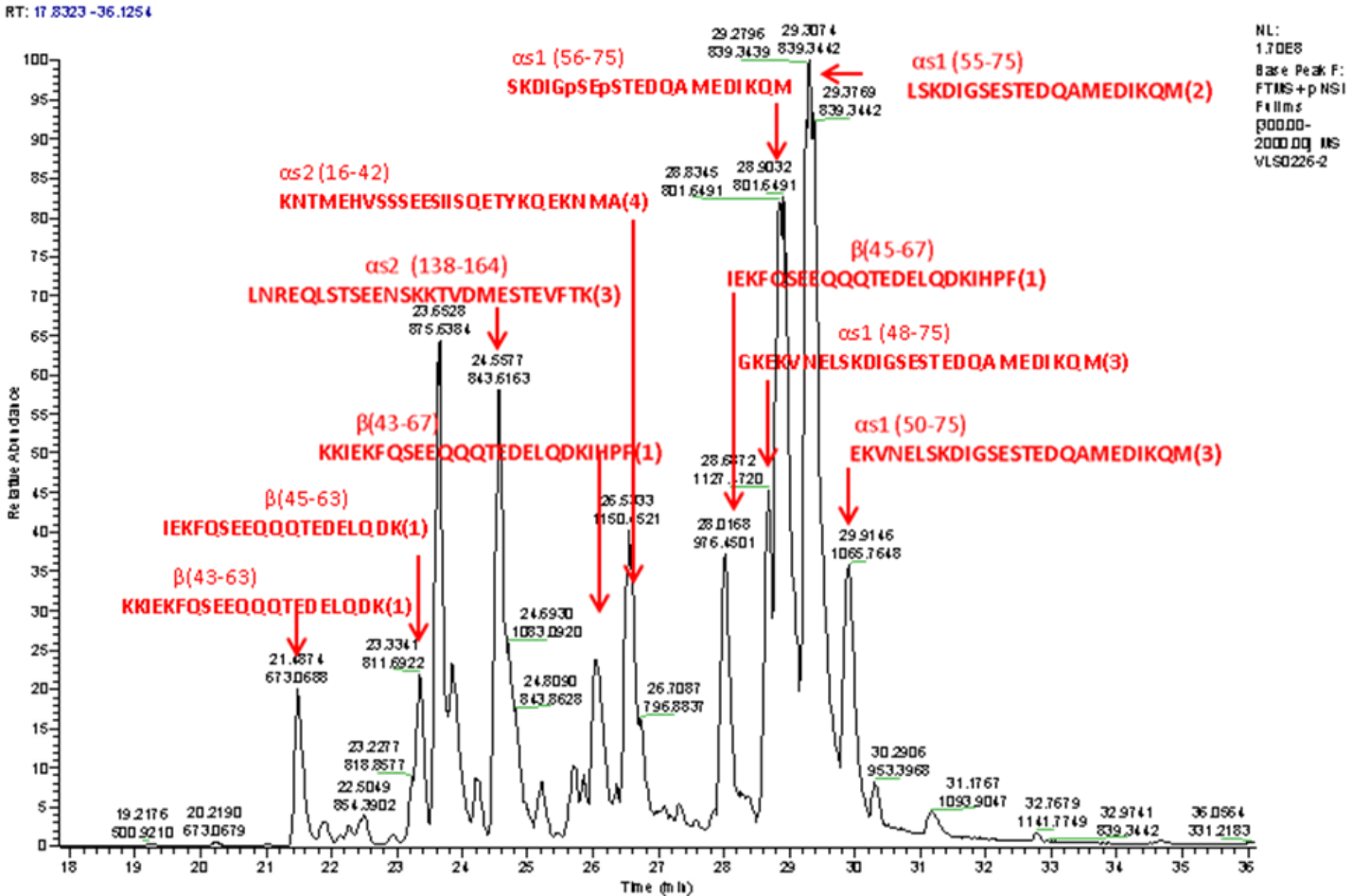
biocompatible, biodegradable, low-toxic, bioadhesive

attract increasing attention in the fields of food, textile, cosmetics, biomedical, pharmaceutical, and other industries.

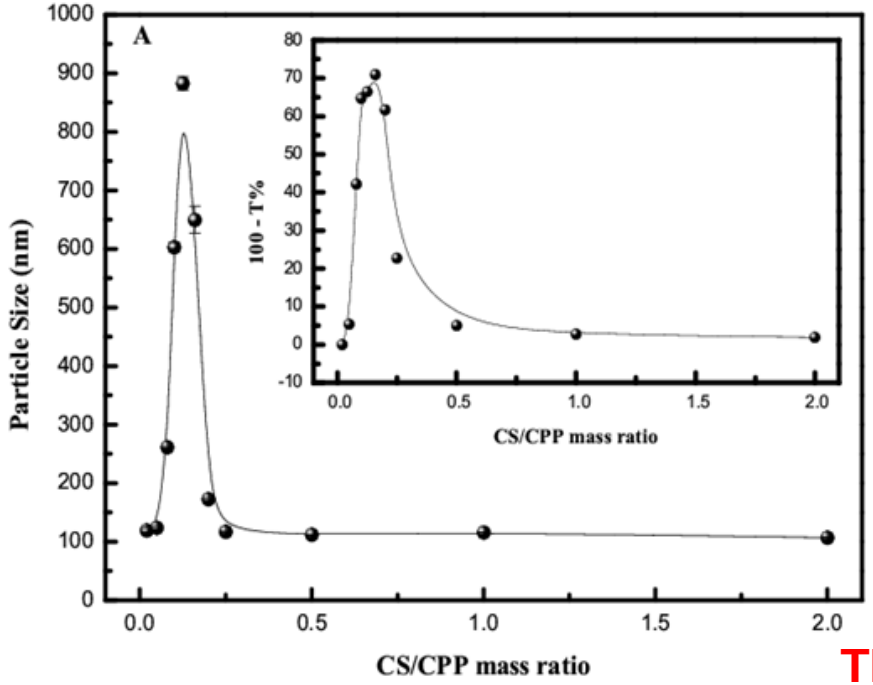
Assembly of Nanoparticles from Caseinophosphopeptides (CPPs) and Chitosan to Improve Calcium Absorption

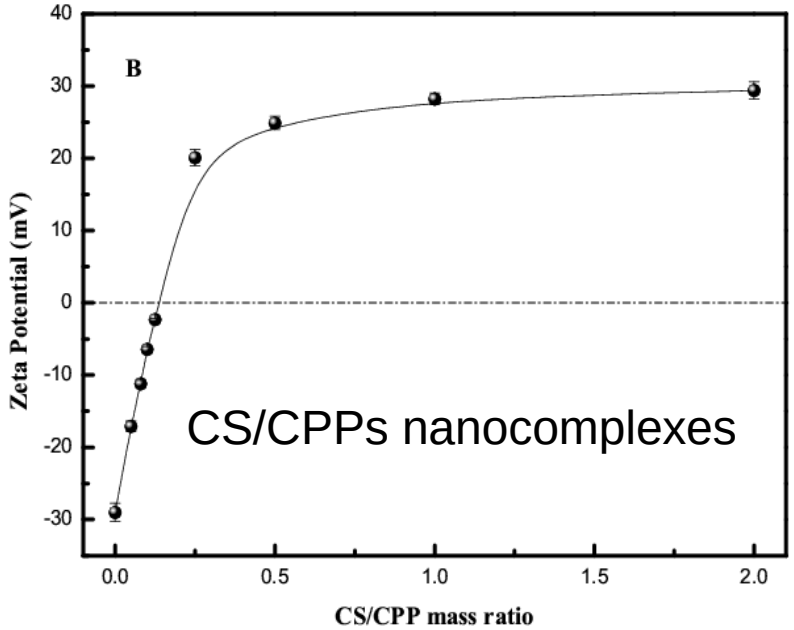
- CPPs are bioactive peptides derived from tryptic digestion of casein;
- CPPs can enhance the mineral absorption/bioavailability in small intestine;
- CPPs contain clusters of phosphorylated seryl residues (see LC-MS data) .

Peptide sequences of CPPs.

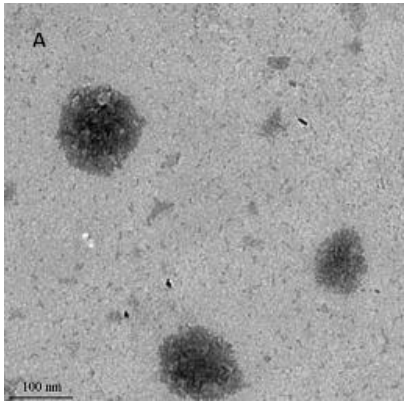


Assembly of Nanoparticles from Caseinophosphopeptides (CPPs) and Chitosan (CS): Effect of CS/CPPs Mass Ratios

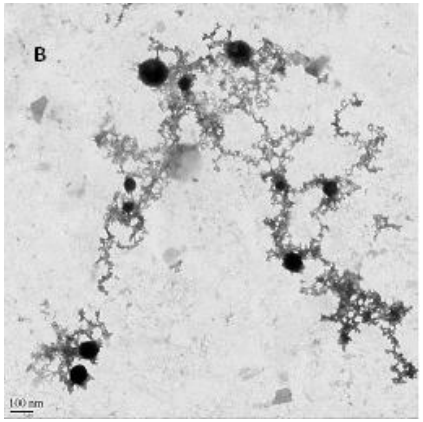




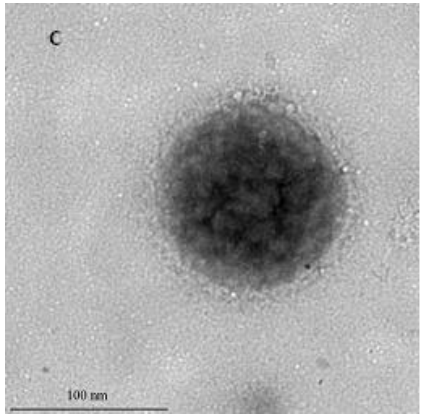
TEM Images



Mass ratio of CS/CPPs=0.20

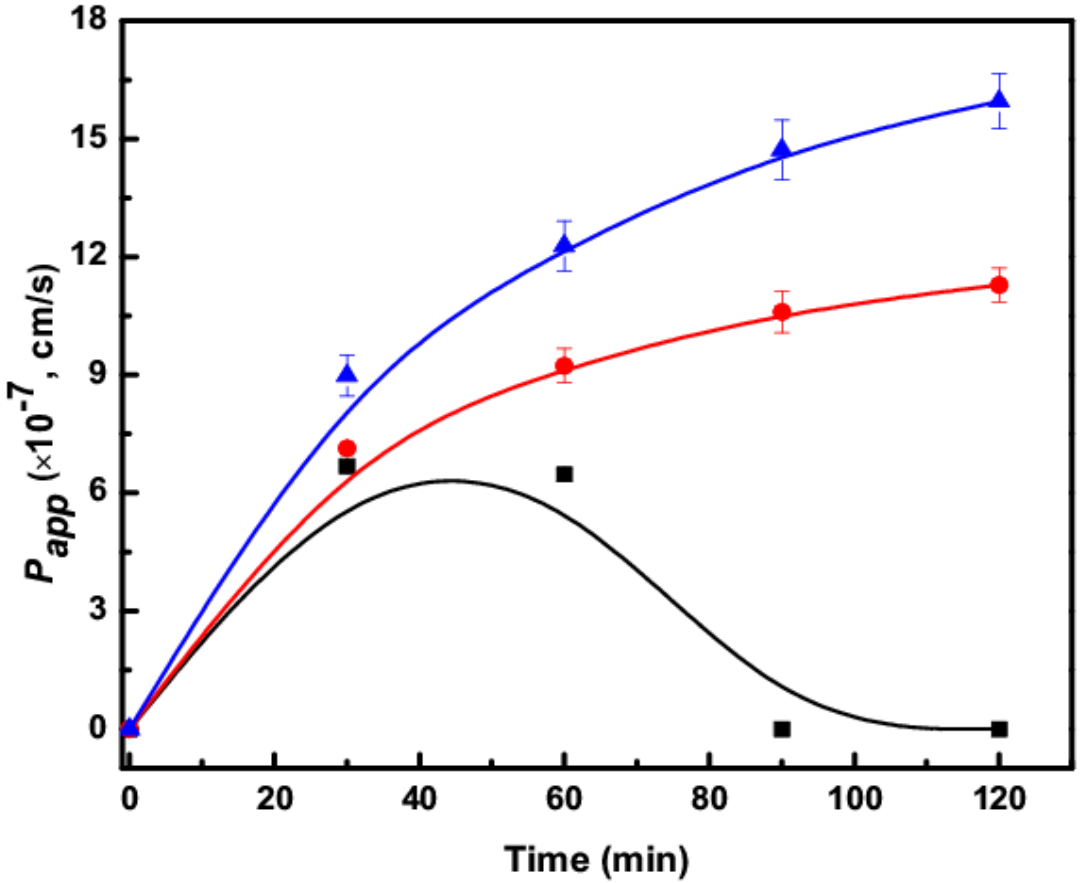


Mass ratio of CS/CPPs=0.25



Mass ratio of CS/CPPs=1.0

Absorption of EGCG in Caco-2 Cells



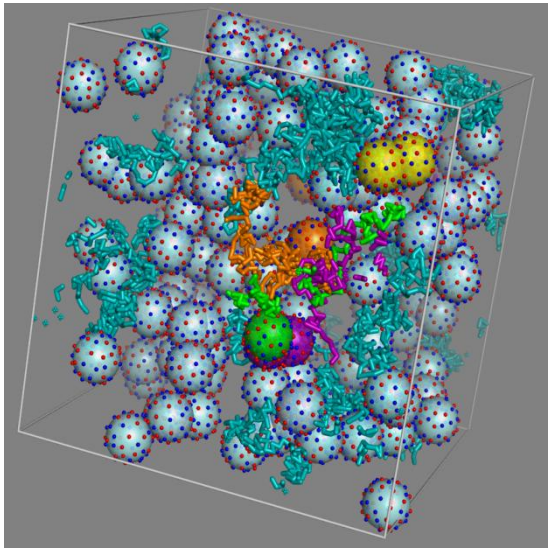
Transport of non-encapsulated (■) and CS-CPP nanoparticles encapsulated EGCG (●,1mg/mL CS; ▲,2mg/mL CS) through Caco-2 cell monolayers

Intellectual Merit

• This project investigates the mechanism which contributes to the assembly of protein/polysaccharide-based particles with the sizes ranging from a few nanometers to several micrometers, nanoscale interactions between proteins and polyelectrolytes, and macroscopic rheological properties of protein/polyelectrolyte coacervates—a theme which is of enormous importance in both fundamental understanding of colloid-polyelectrolyte interactions and industrial applications ranging from drug/gene delivery, cosmetics, functional food, dietary supplements, protein separation, to biosensors.

• Provide experimental basis for further theoretical studies.

-Right figure is a Snapshot of our recent Monte Carlo simulation configuration at pH 5.0.



Broader Impact

- Economic impact

- Broad applications in the formulation of multicomponent functional foods, drug, personal care products, waste water management etc.

- Student training

- International collaboration

- Dissemination

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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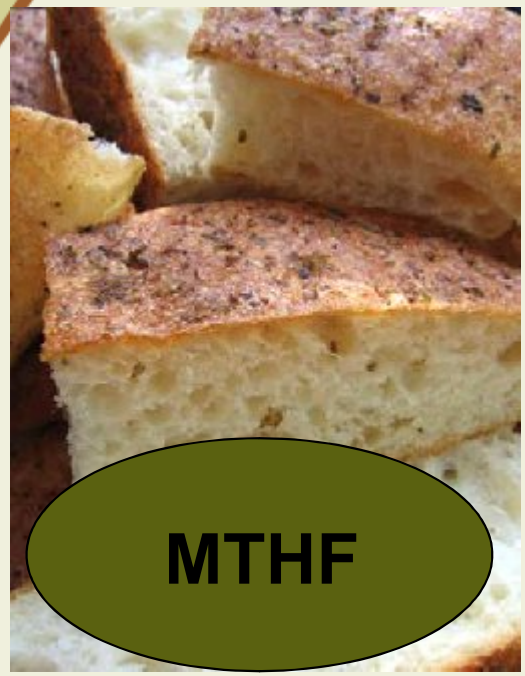
Folate fortification



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**

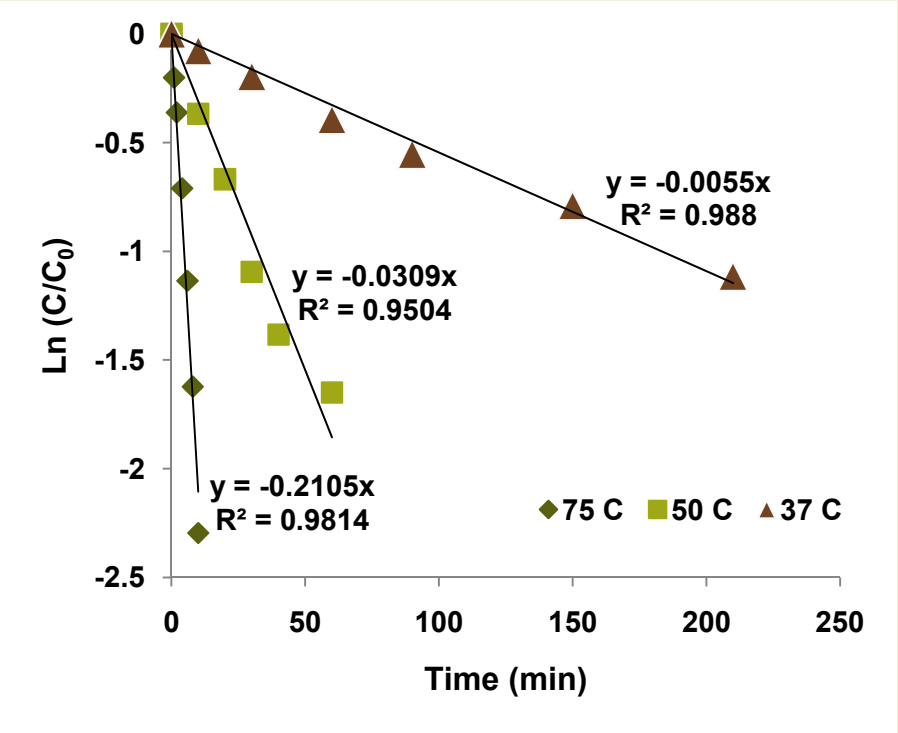


Fig 1. Thermal degradation kinetics of MTHF (50µg/ml, pH 6.8, limited O_2)

- **Food system**

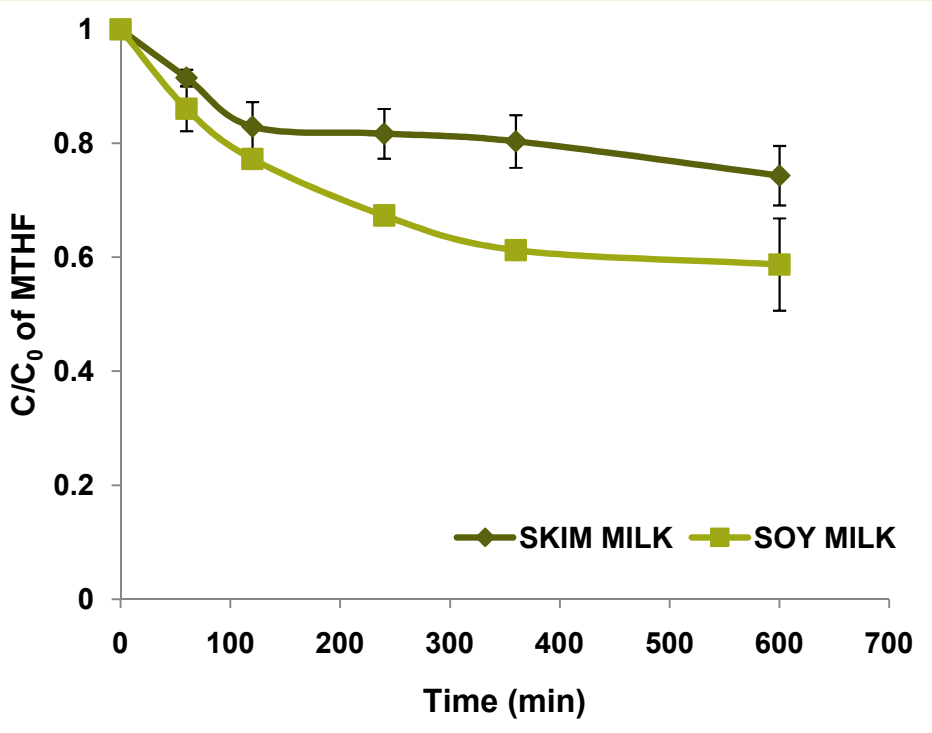


Fig 2. Degradation of MTHF in skim milk and soy milk (50ºC)

Interactions between antioxidants and MTHF

- **Improvement of thermal stability**

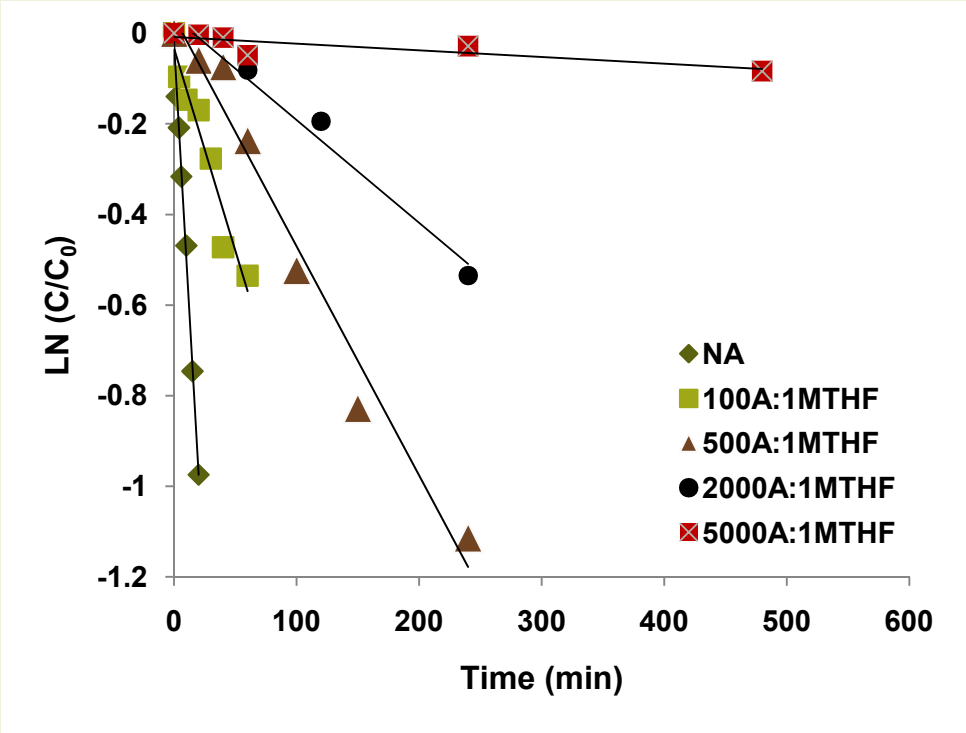


Fig 3. Thermal degradation of MTHF containing different molar ratios of sodium ascorbate (A) (50ºC, pH 6.8)

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

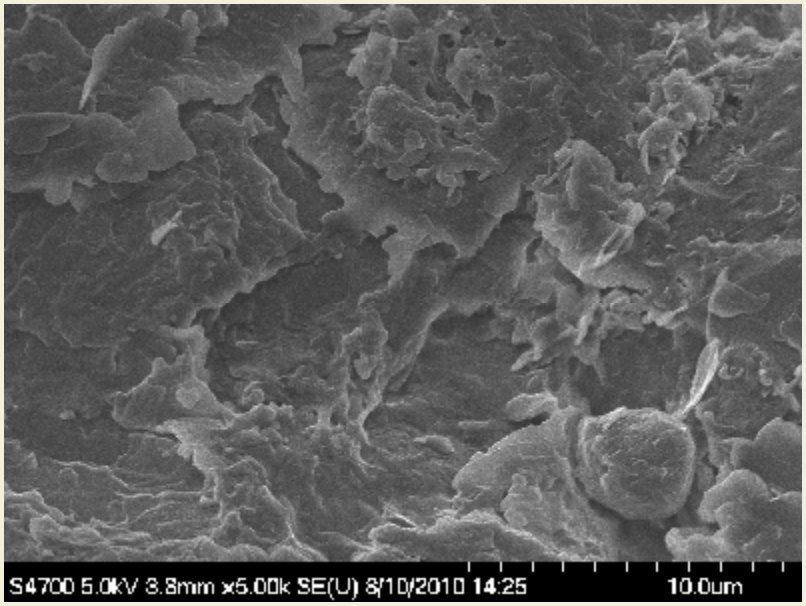
Tab 1. Regeneration of MTHF by NaAc

	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

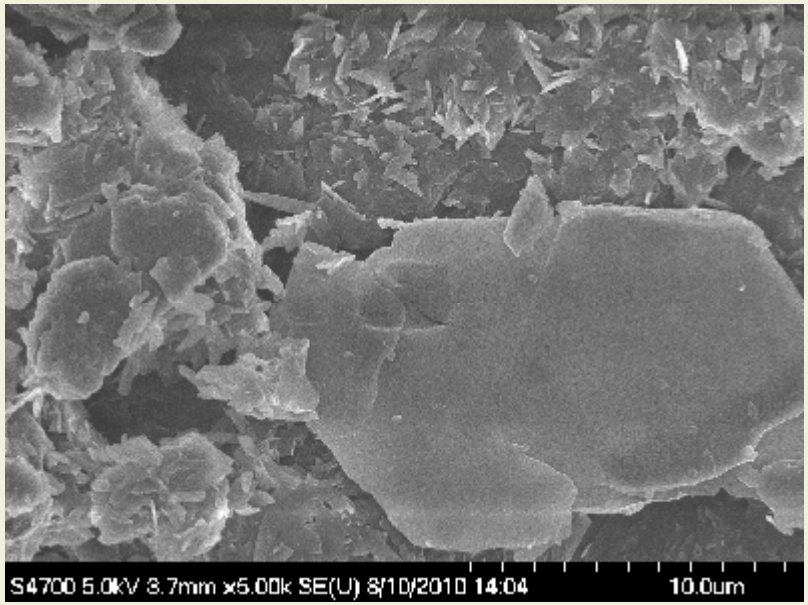
* MTHF was heated at 50ºC for 60min and 150min, then antioxidants were added. Data are expressed as C/C_0

Stabilization of MTHF using microencapsulation

- **Electronmicroscope pictures**



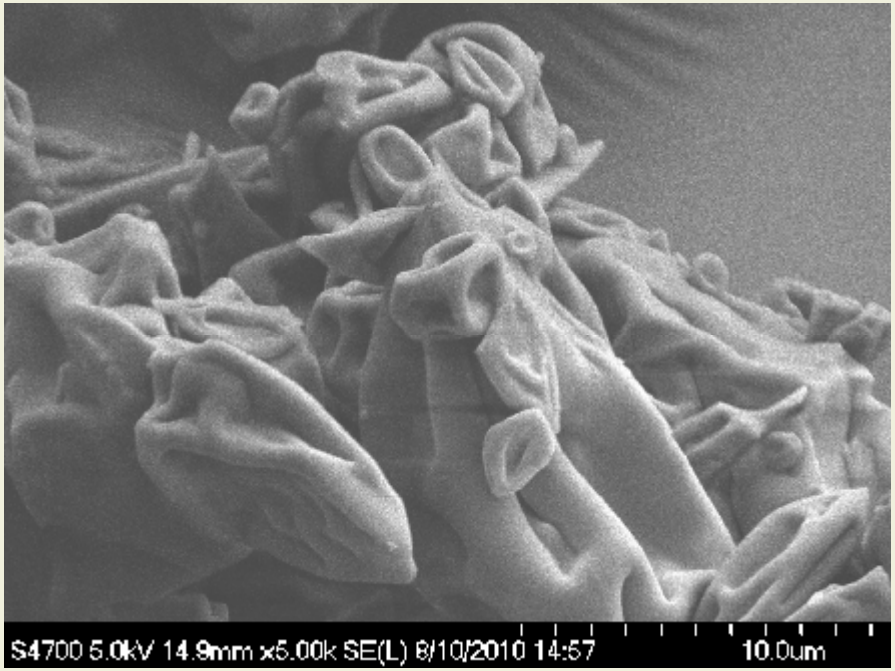
Free MTHF



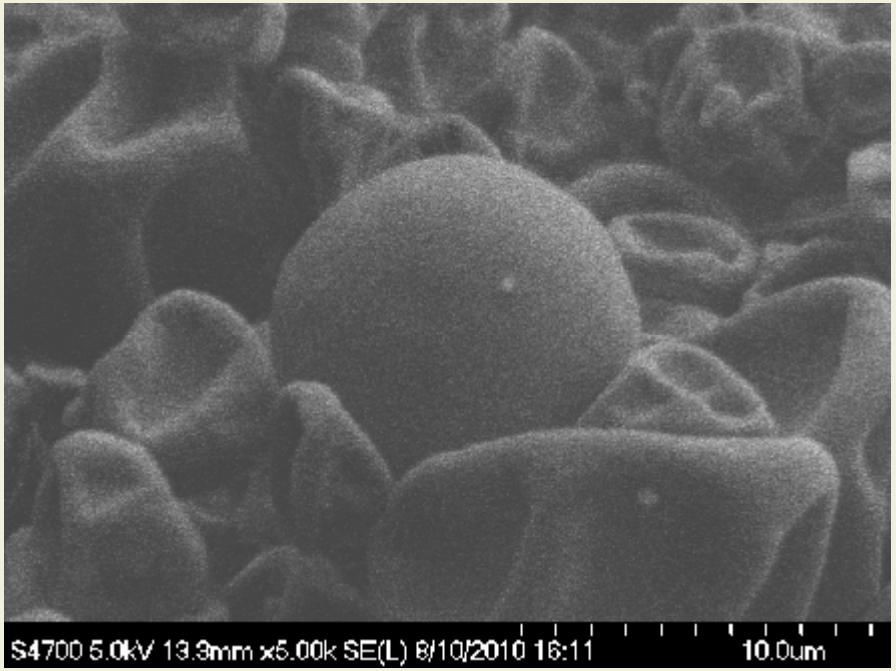
Stearate coating

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**

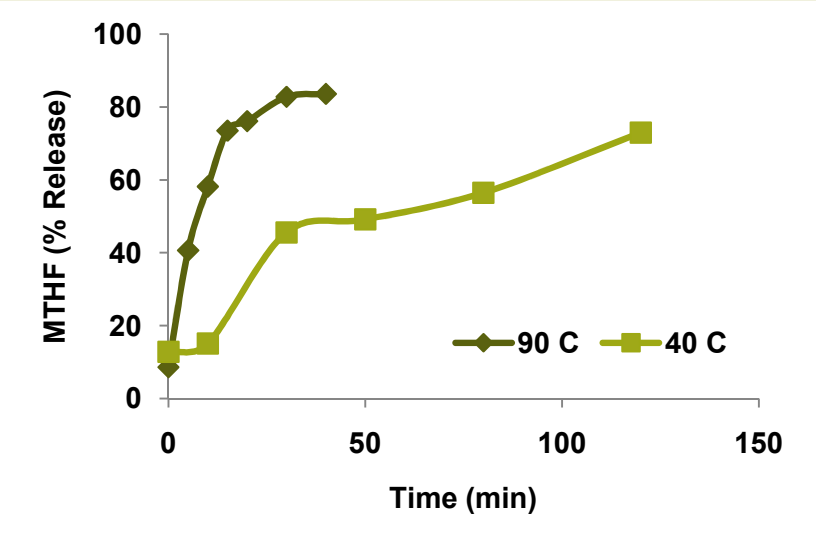

Fig 4. Release of MTHF from stearate coating to the buffer system

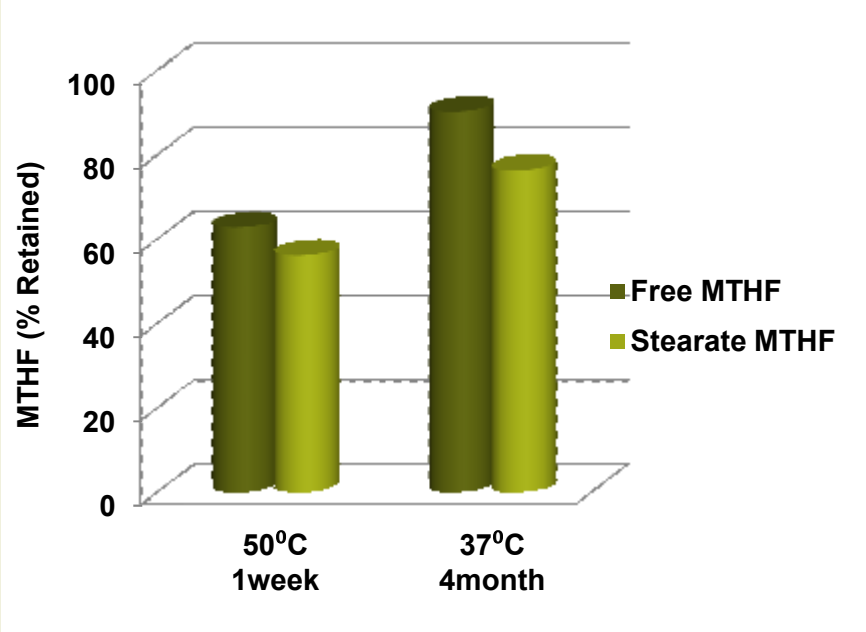

Fig 5. Stability of free and encap MTHF (dry form)

Stearate MTHF

- **Food system**

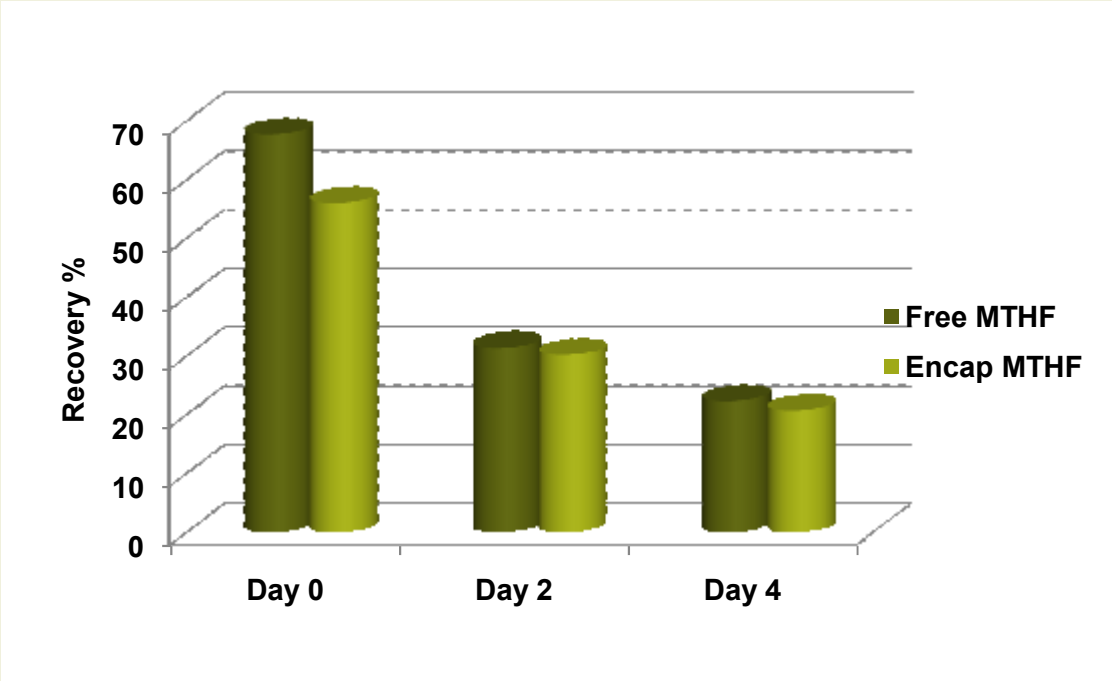

Fig 6. Recovery of MTHF in bread (after baking and during storage at RT)

PS MTHF & PS (MTHF+AA)

- **Food system**

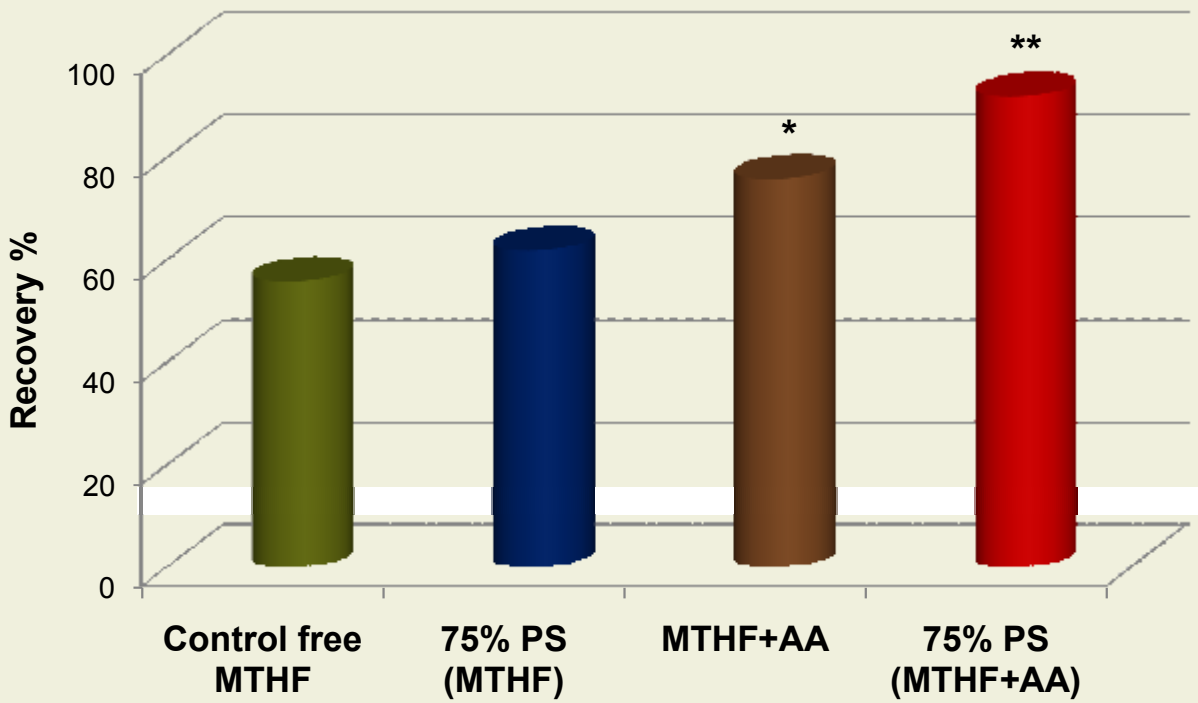

Fig 7. Recovery of MTHF in bread after baking

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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Reference

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- Venn BJ, Green TJ, Moser R, Mann JI. Comparison of the effect of low-dose supplementation with L-5-methyltetrahydrofolate or folic acid on plasma homocysteine: a randomized placebo-controlled study. Am J Clin Nutr 2003;77:658-62.

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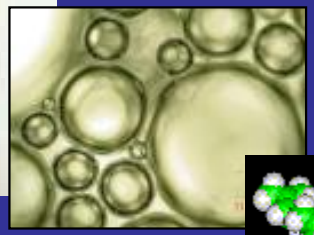
Design Of Nano-Laminated Coatings to Control Bioavailability of Lipophilic Food Components

D. Julian McClements, E. Decker, Y. Park

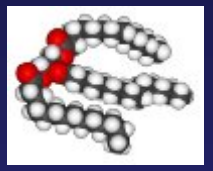
Department of Food Science

University of Massachusetts , Amherst

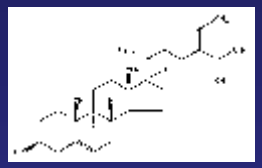


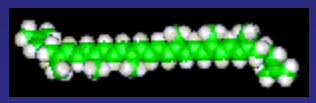
Problem Addressed: Stabilization & Delivery of Bioactive Lipophilic Components

Functional & Bioactive Lipids



- Essential oils (*e.g.*, citral, limonene, eugenol)
- Unsaturated Fats (*e.g.*, ω-3, CLA)
- Phytosterols & Phytostanols (*e.g.*, Sitostanol)
- Carotenoids (*e.g.*, lycopene, β-carotene)





- Antioxidants
- Antimicrobials
- Anti-inflammatory agents
- Chemo-preventive agents
- Anti-Heart disease
- Anti-Diabetes
- ……

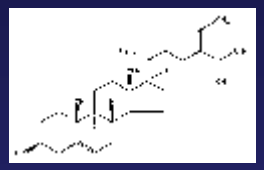
Challenges:

- Low water solubility
- Chemical instability
- Crystalline
- Low bioavailability
- Poor matrix compatibility

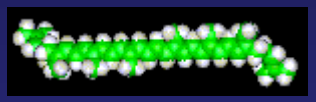
Overall Objective: To Develop Delivery Systems to Control Lipophilic Component Bioavailability

Increasing Bioavailability

- Beneficial Bioactive Components
- Lipophilic actives, Minerals



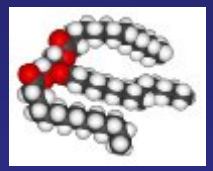
Phytosterols



Carotenoids

Decreasing Bioavailability

- Detrimental Bioactive Components
- Saturated fats, cholesterol

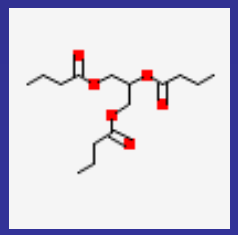


Triacylglycerols

Cholesterol

Controlling Release

- Site-specific Bioactive Components
- Flavors, Anticancer agents, Satiety controllers



Tributyrin

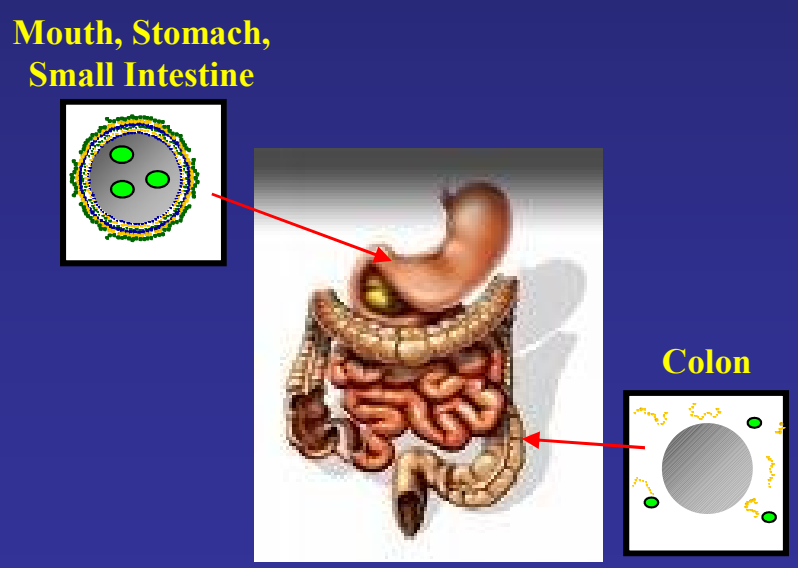
Controlled Bioavailability:

Designing Systems to Control Lipid Digestion

Targeted Release

- **Tunable stability/instability profiles**
  - *Encapsulation and release of functional components in response to specific environmental triggers (pH, enzymes, I)*

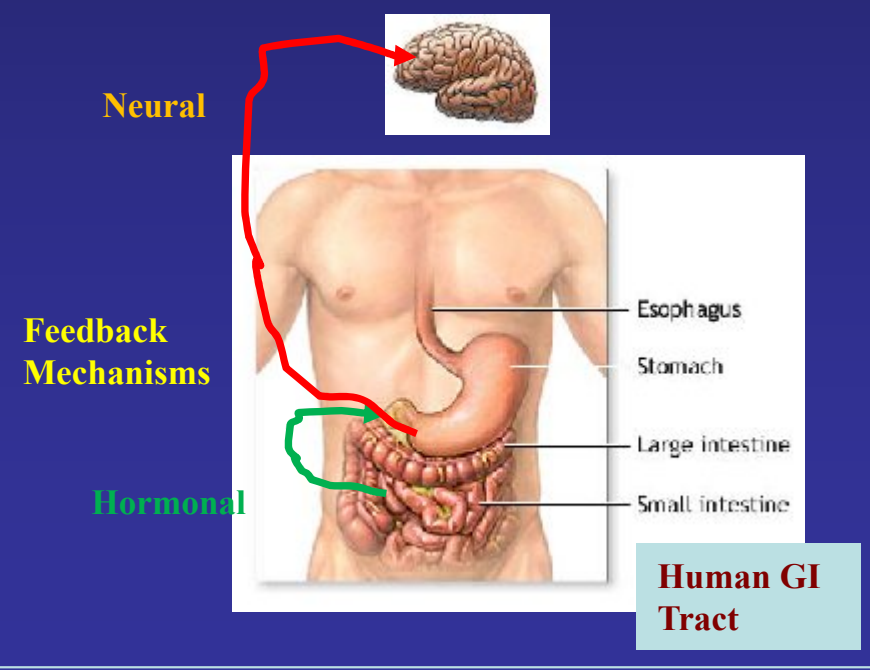
- Deliver biologically active components to site of action: mouth, stomach, small intestine or colon



Modulating Satiety

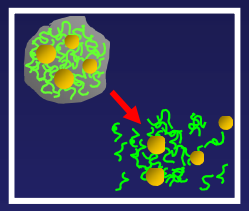
- **Acid stable foods**
  - *Even distribution of fat in stomach*
- **Delay digestion**
  - *Deliver more undigested nutrients to ileum*

- Generate neural & hormonal signals that enhance satiety, thereby reduce amount of food consumed

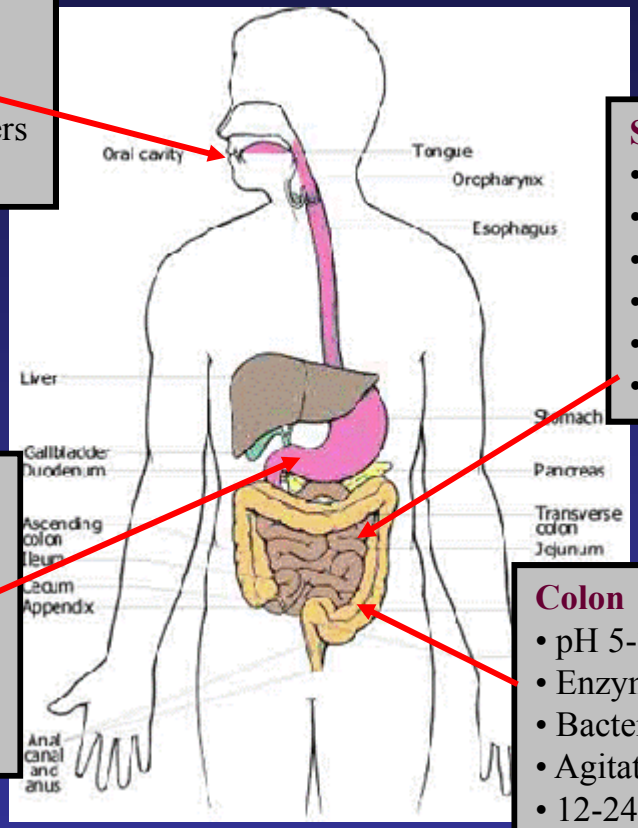


Background: Understanding Fate of Ingested Bioactive Lipophilic Components in Human Body

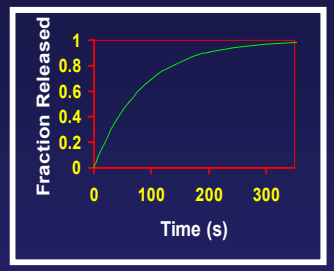
Food Matrix Disruption



Mouth
- pH 5-7
- Enzymes
- Salts
- Biopolymers
- 5 – 60 s

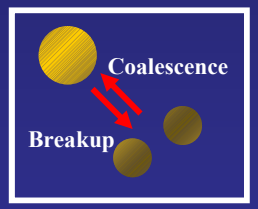


Small Intestine
- pH 6-7.5
- Enzymes
- Salts, Bile
- Biopolymers
- Agitation
- 1 – 2 hours

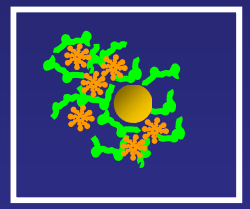


Accumulation at site of action

Droplet Formation & Disruption

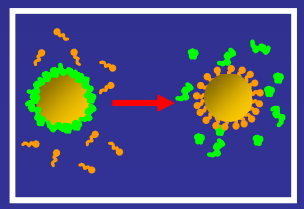


Stomach
- pH 1-3
- Enzymes
- Salts
- Biopolymers
- Agitation
- 30 min – 4 hours

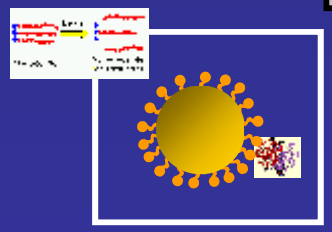


Binding & Interactions

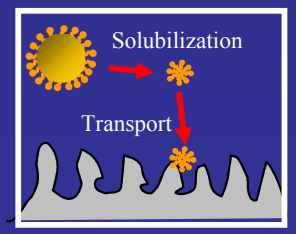
Colon
- pH 5-7
- Enzymes
- Bacteria
- Agitation
- 12-24 hours



Competitive Adsorption & Displacement

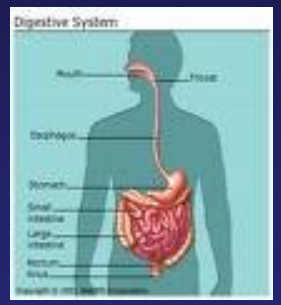


Enzyme Adsorption & Activity



Solubilization & Transport

Challenges: Designing Viable Food-Grade Delivery Systems



Matrix Compatibility
- Optical
- Rheological
- Stability
- Flavor

Processing
- Heating
- Cooling
- Drying
- Shearing

Storage
- Temperature
- Mechanical stress
- Light
- Oxygen
- Time

Consumption
- Appearance
- Texture
- Taste and Aroma
- Convenience

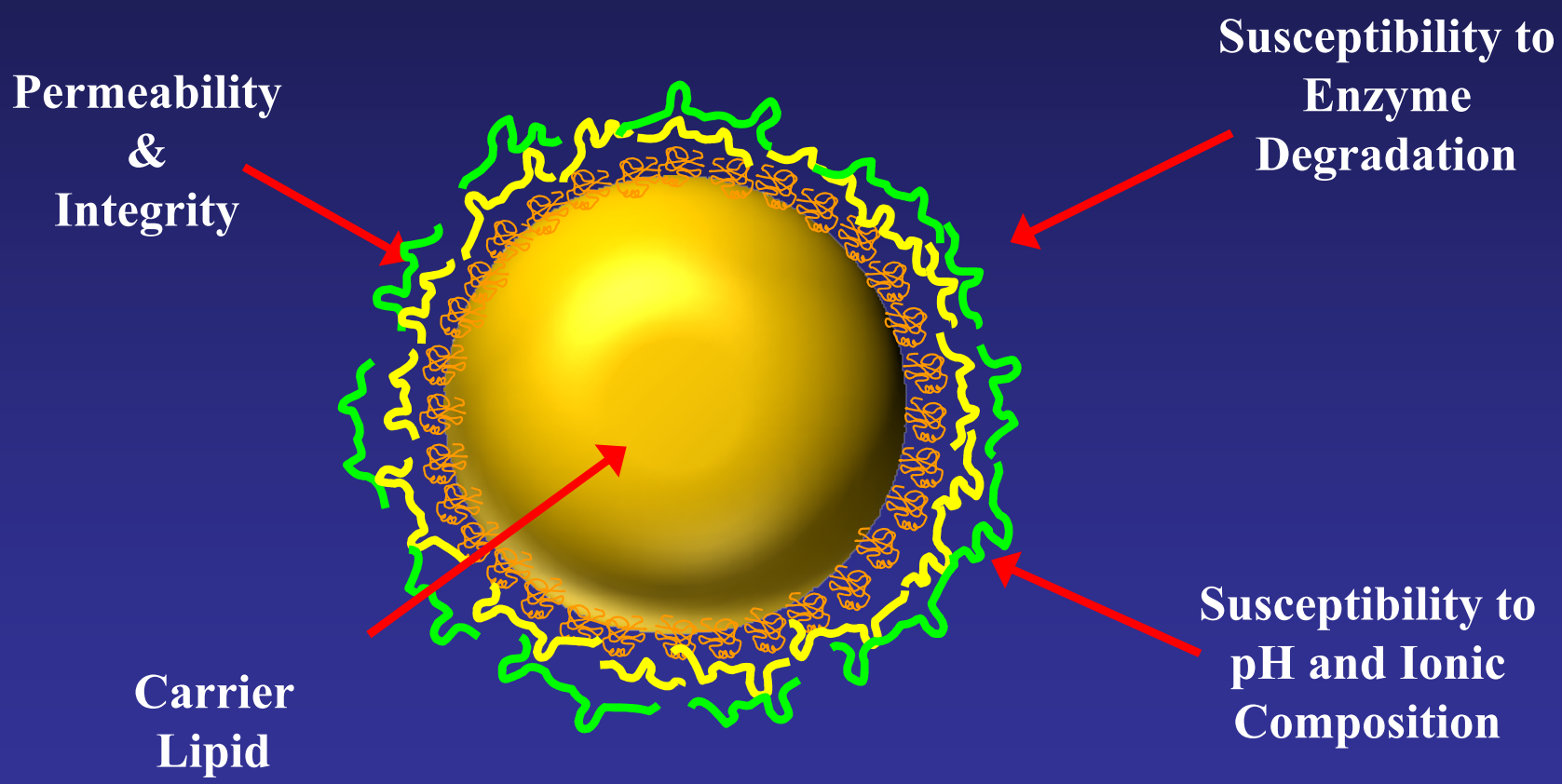
Ingestion
- Digestion
- Absorption

Stable

Unstable

DELIVERY SYSTEM CRITERIA:
- Fabricated from food grade ingredients using economic processing operations
- Designed to function over wide range of conditions in food product and human body
- Sensory acceptance

Proposed Solution: Nanolaminated Emulsion Droplets to Encapsulate & Deliver Lipophilic Bioactives



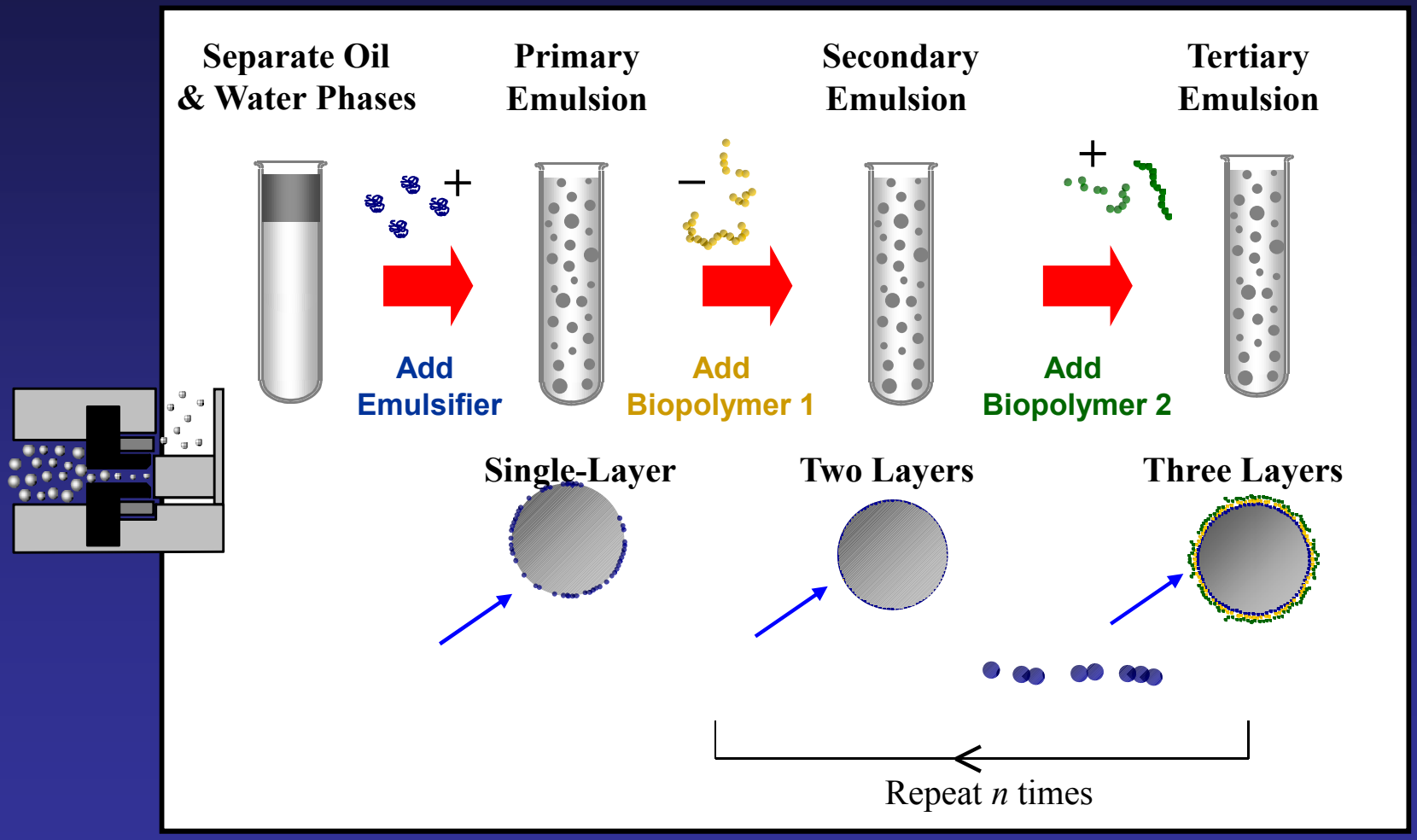
Specific Objectives:

Controlling Delivery of Bioactive Lipophilic Components

- **(1). In vitro Studies:** Determine role of nanolaminated coating properties (thickness, charge, structure) on *in vitro* digestibility/release of lipids.
  - **Design Delivery Systems with Specific Release Characteristics**

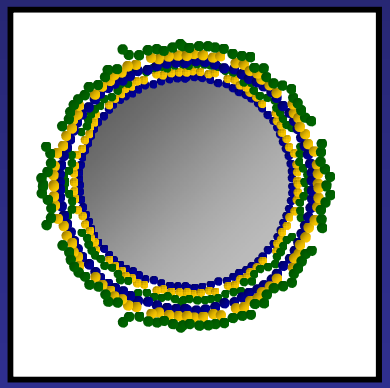
- **(2). In vivo Studies:** Test designed delivery systems using animal feeding studies.
-

Nanolaminated Emulsion Droplets:
Formation using LbL Method



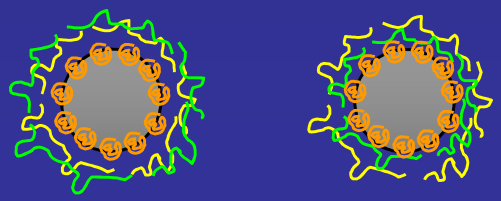
Project Achievements: *In Vitro* Studies of Specific Biopolymer Characteristics

- **Composition of Layers:** The nature of the biopolymers used determines physical stability, chemical stability, digestibility, and release
  - Casein, Whey protein, Lactoferrin, Chitosan, Pectin, Alginate, Chitosan.



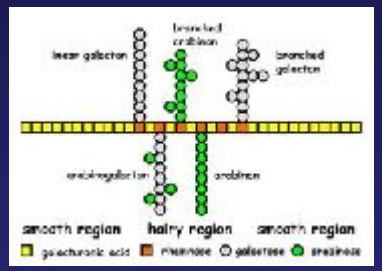
- **Number of Layers:** Increasing the number of layers usually increases the physical and chemical stability, but decreases the rate of digestibility.

- **Structure of Layers:** The structural organization of the different layers in the coatings also impacts their functionality.

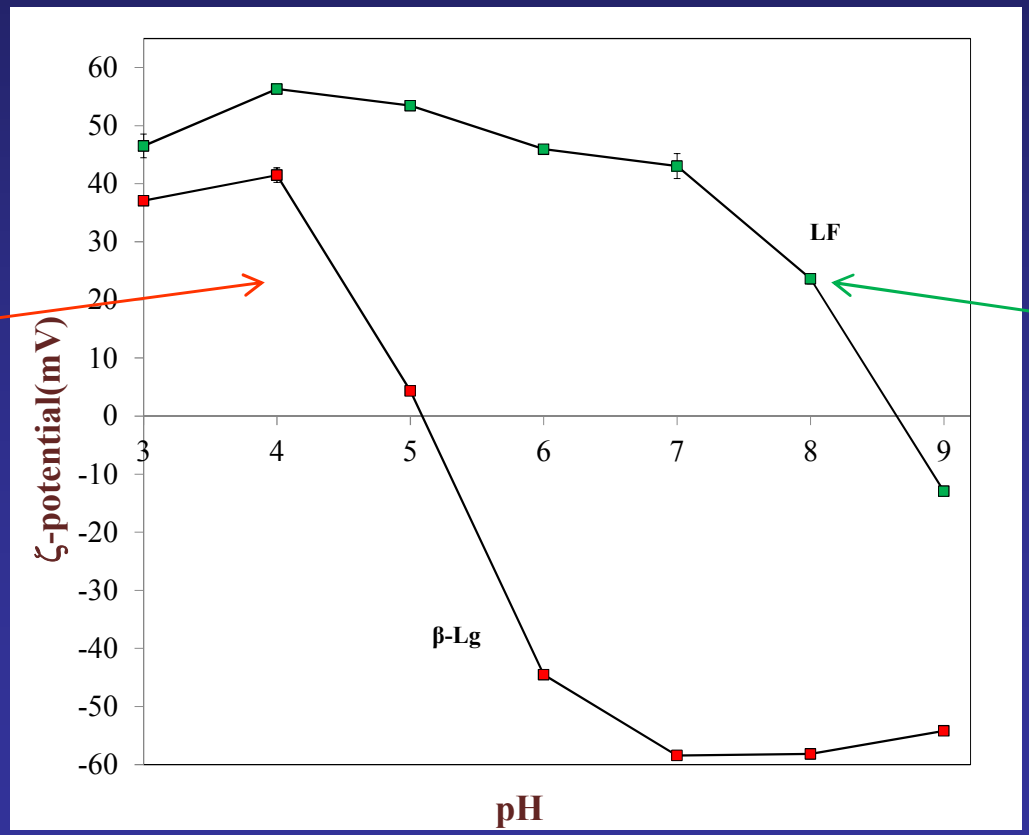


Preparation of Nanolaminated Coated Droplets:
Formation using Proteins

Previous research used indigestible polysaccharides (dietary fiber) to cover lipid droplets with nanolaminated coatings
- Can digestible proteins be used also?



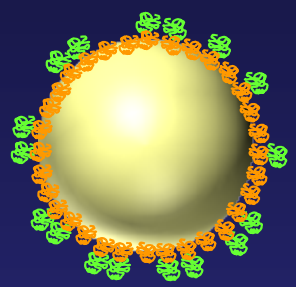
β-Lactoglobulin
(pI = 5)

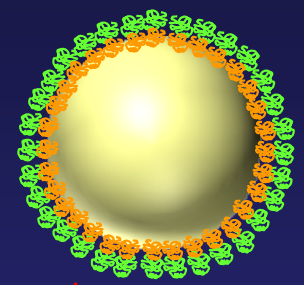


Lactoferrin
(pI = 8.5)

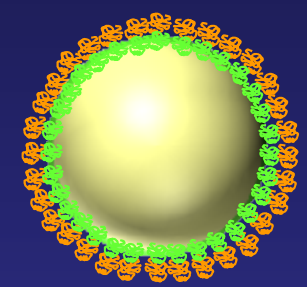
Preparation of Nanolaminated Coated Droplets:

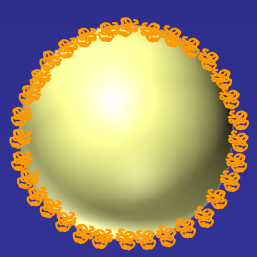
Multilayer Protein Coatings

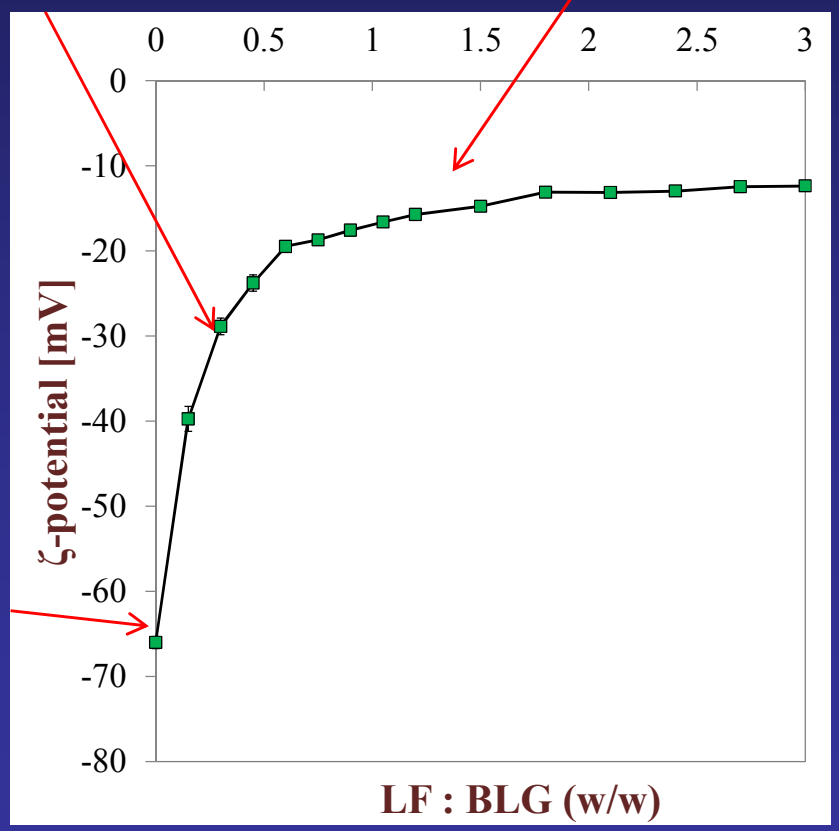




Multilayer protein coatings can be formed



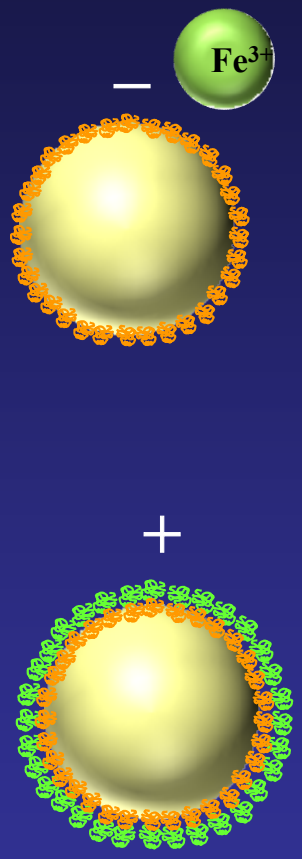


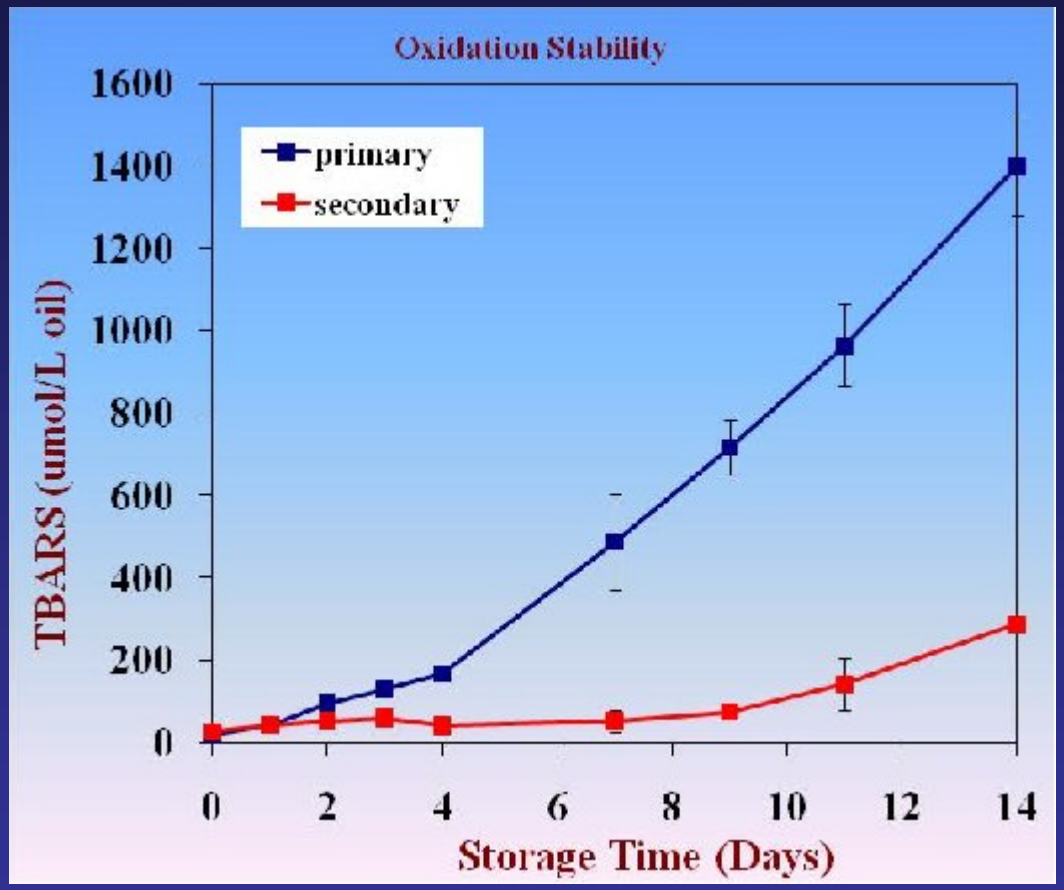


Tunable Layer Properties:

- Composition
- Structural Organization
- Number

Functionality of Nanolaminated Coated Droplets: Improving Chemical Stability

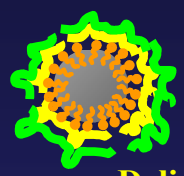




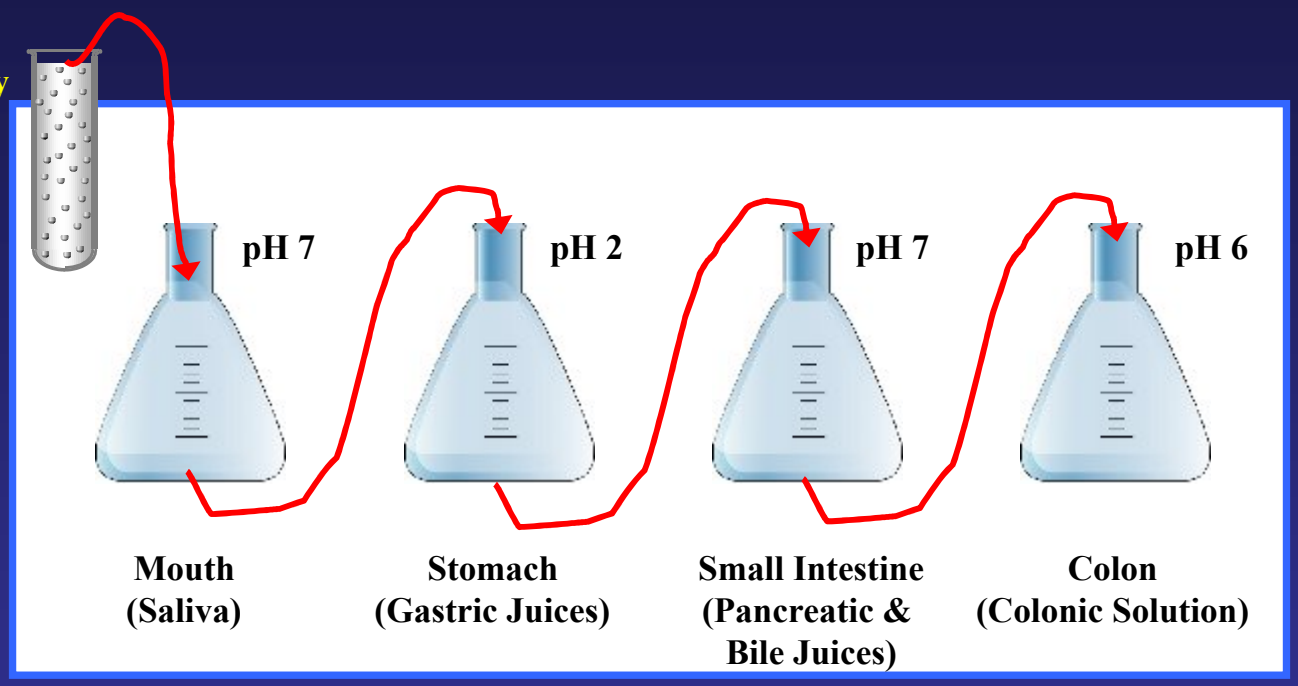
Caseinate-Lactoferrin Stabilized Emulsions (pH 7)

Bioactivity Studies:

Testing Lipid Digestion & Bioactivity



Delivery System



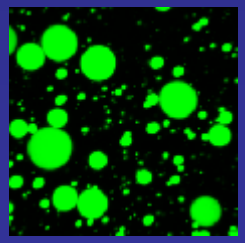
Digestion & Release

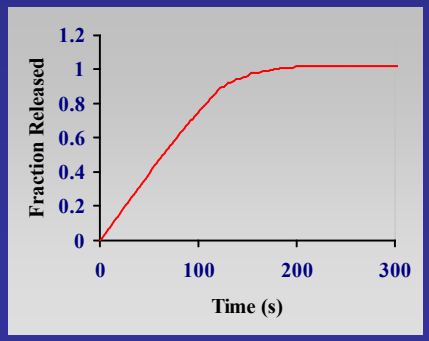
Analytical Methods:
- Particle Size
- ζ-Potential
- Microstructure
- Chemical Methods
- Feeding Studies

Bioactivity

Feeding Studies

Structure





In vitro Studies

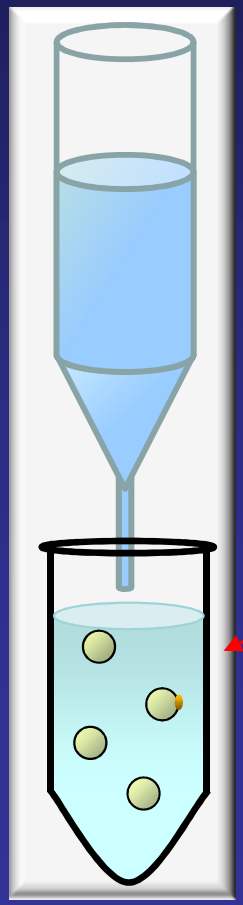
In vitro Testing of Lipid Digestibility

Development of Standard pH Stat Method

TAG

MAG + 2FFA

NaOH

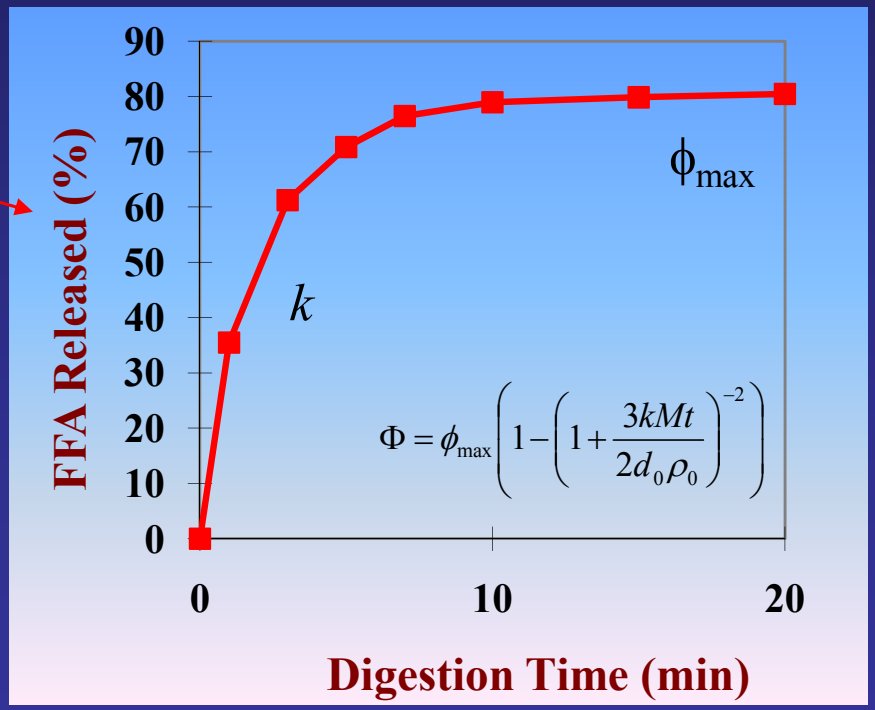


pH 7.0
Simulated Small Intestinal Fluid

pH-Stat Titration

- Sample
- Enzymes: Lipase, Protease
- Bile salts/phospholipids
- Salts: Calcium, Sodium

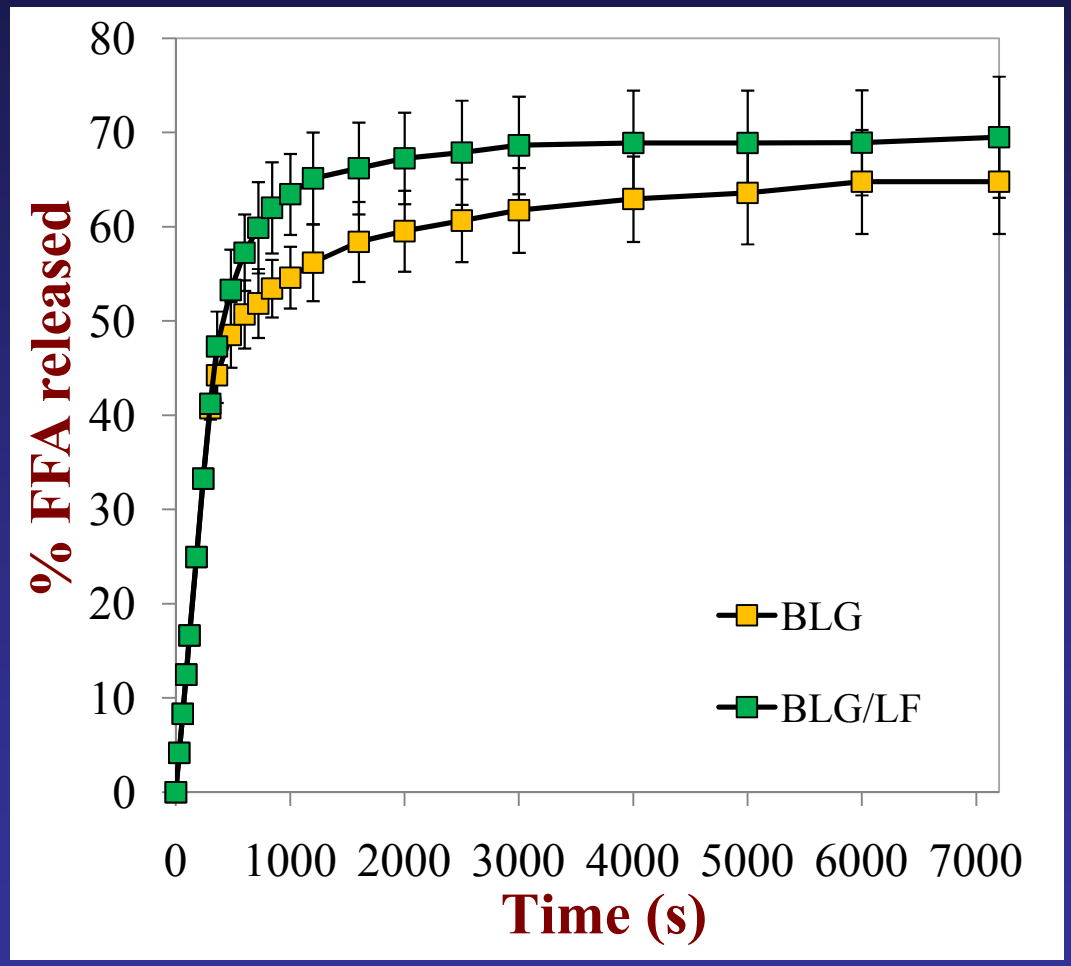
Digestion Rate & Extent

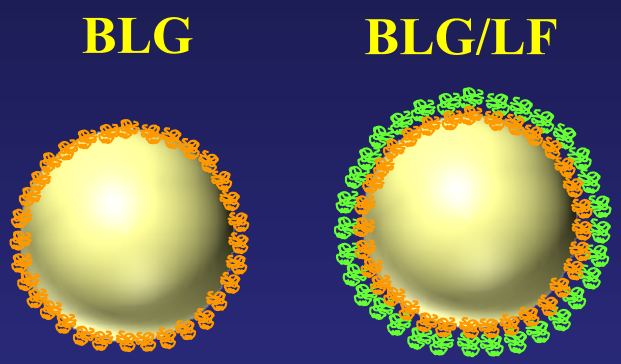


Highly Dependent on SSIF composition: Bile, Lipase, Calcium

Preparation of Nanolaminated Coated Droplets:

Multilayer Protein Coatings

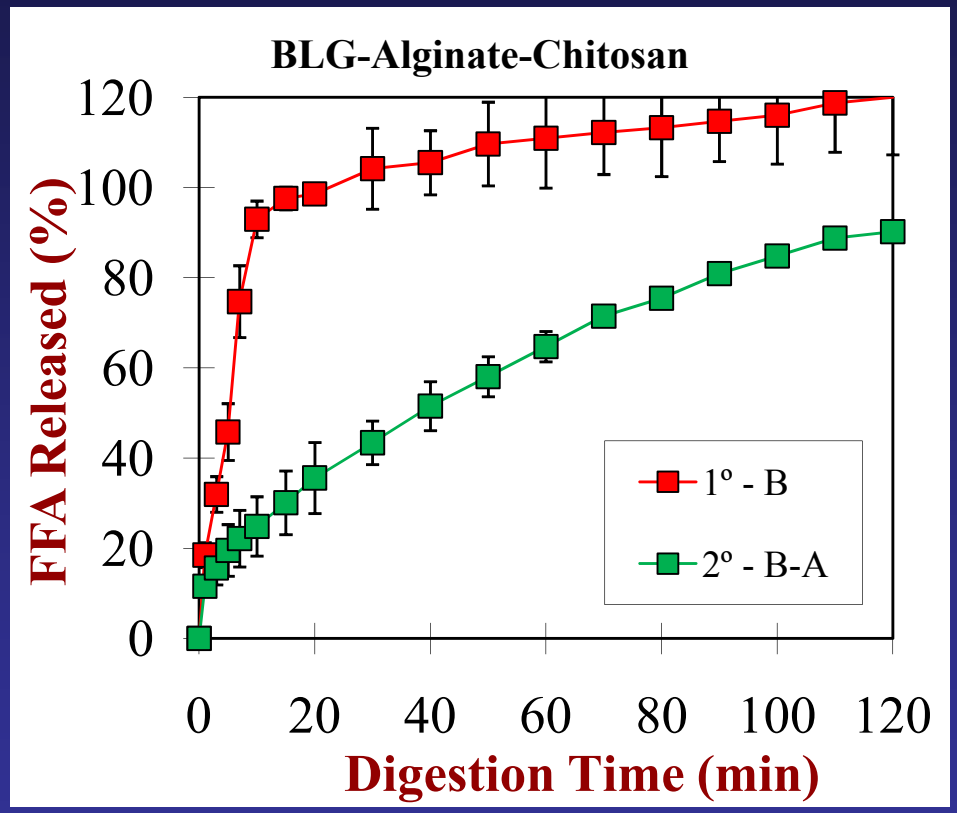




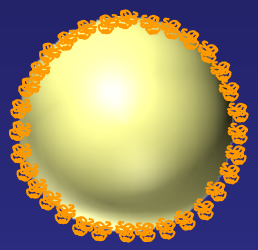
Protein coatings do not greatly inhibit lipid digestion

Applications of Multilayer Emulsions

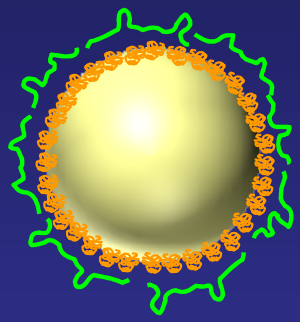
Digestibility in *In Vitro* Model



BLG



BLG/Alginate



Digestion depends on multilayer properties

5 mg/ml bile, 1.6 mg/ml lipase, 20 mM calcium

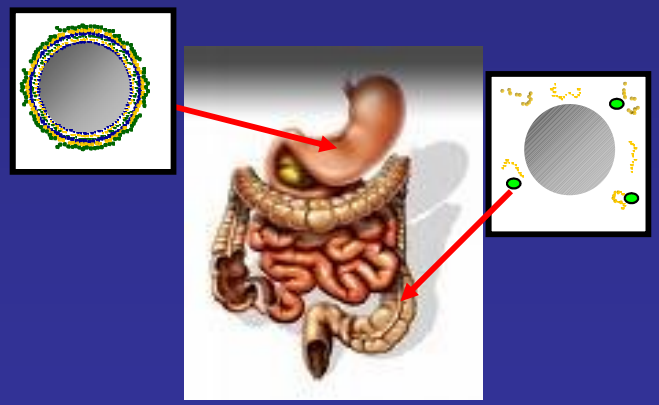
Proposed Further Work

Design Functional Delivery Systems

- **Digestible System**
- **Indigestible System**
- **Controlled Release System**

In vivo Testing of Delivery Systems:

- **Animal Feeding Studies**



Rational Design of Delivery Systems:
Establishing Guiding Principles

- Polarity
- Physical State
- Stability
- Bioactivity

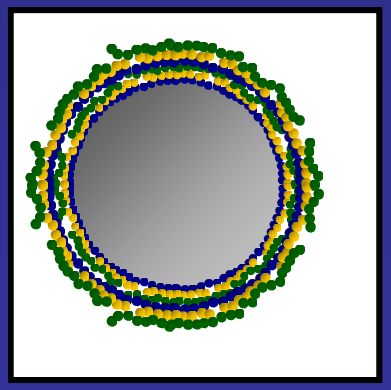
Specify Active Component Properties & Challenges

Specify Food Matrix Properties

- Optical
- Rheological
- Flavor
- Stability

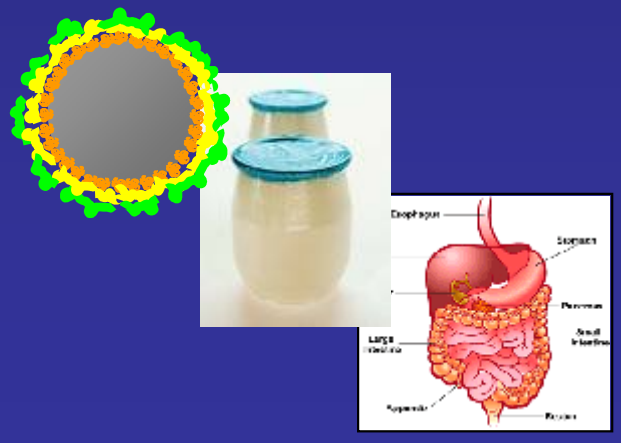
- Layer composition
- Layer number
- Layer structure
- Layer functionality

Design Delivery Systems



Conclusions

- Nanolaminated biopolymer coatings have been shown to have great potential for encapsulating lipids and altering their bioavailability
- Our research shows that specific coating characteristics can be engineered to control lipid digestion and release
- Systems need to be tested using *in vivo* studies – animal models
- Structured delivery systems must be effective, but also economically viable and compatible with food systems



Selected Publications

1. McClements DJ, Li Y (2010). Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. ADVANCES IN COLLOID AND INTERFACE SCIENCE 159,2,213-228.
2. Li Y, Hu M, Xiao H, McClements DJ (2010). Controlling the functional performance of emulsion-based delivery systems using multi-component biopolymer coatings. EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS 76,1,38-47 .
3. Tokle T, Lesmes U, McClements DJ (2010). Impact of Electrostatic Deposition of Anionic Polysaccharides on the Stability of Oil Droplets Coated by Lactoferrin JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 58,17,9825-9832 ..
4. Chen BC, McClements DJ, Gray DA, Decker, EA. (2010). Stabilization of Soybean Oil Bodies by Enzyme (Laccase) Cross-Linking of Adsorbed Beet Pectin Coatings . JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 58,16,9259-9265 AUG 25 2010
5. Lesmes U, Baudot P, McClements DJ (2010). Impact of Interfacial Composition on Physical Stability and In Vitro Lipase Digestibility of Triacylglycerol Oil Droplets Coated with Lactoferrin and/or Caseinate . JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 58,13,7962-7969.
6. Li Y, McClements DJ (2010). New Mathematical Model for Interpreting pH-Stat Digestion Profiles: Impact of Lipid Droplet Characteristics on in Vitro Digestibility. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 58,13,8085-8092 .
7. Gudipati V, Sandra S, McClements DJ, Decker, E.A. (2010). Oxidative Stability and in Vitro Digestibility of Fish Oil-in-Water Emulsions Containing Multilayered Membranes . JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 58,13,8093-8099
8. McClements DJ. (2010). Emulsion Design to Improve the Delivery of Functional Lipophilic Components . ANNUAL REVIEW OF FOOD SCIENCE AND TECHNOLOGY, VOL 1 Book Series: Annual Review of Food Science and Technology .
9. Lesmes U, Sandra S, Decker EA, McClements DJ . (2010). Impact of surface deposition of lactoferrin on physical and chemical stability of omega-3 rich lipid droplets stabilised by caseinate. FOOD CHEMISTRY 123,1,99-106/
10. Cho YH, Decker EA, McClements DJ . (2010). Formation of Protein-Rich Coatings around Lipid Droplets Using the Electrostatic Deposition Method LANGMUIR 26,11,7937-7945 .
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12. Lesmes U, McClements DJ (2009). Structure-function relationships to guide rational design and fabrication of particulate food delivery systems. TRENDS IN FOOD SCIENCE & TECHNOLOGY 20,10,448-457.
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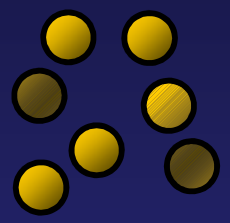
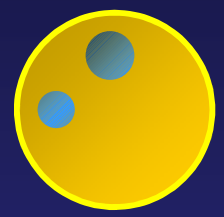
Emulsion-based Delivery Systems

Nano-droplets

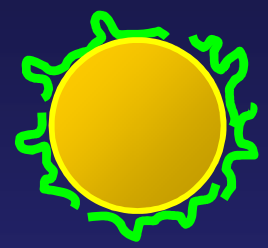


Micro-Droplets

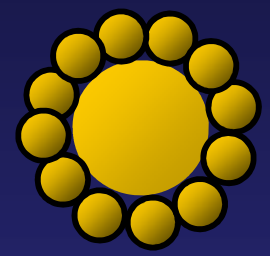
Filled Droplets



Coated Droplets

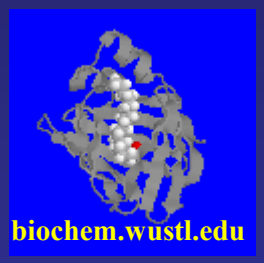


Colloidomes



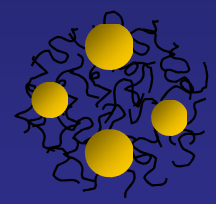
Solid Lipid Particles

Molecular Complexes

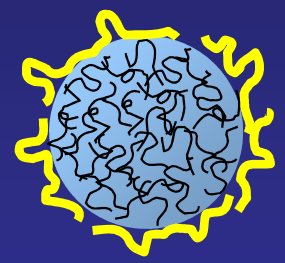


Hydrogel Beads

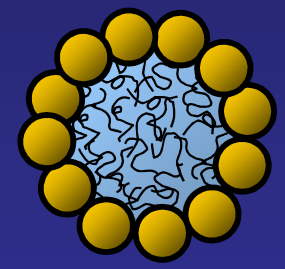
Filled Beads



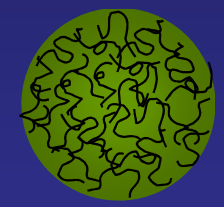
Coated Beads



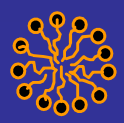
Decorated Beads



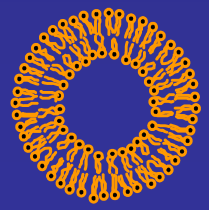
Solid particles



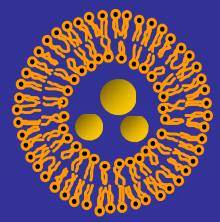
Micelles & Microemulsions



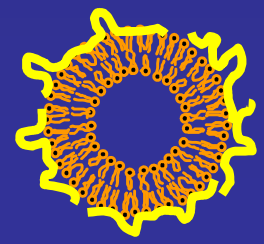
Liposomes



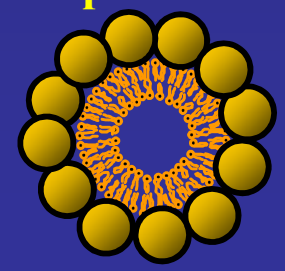
Filled Liposomes



Coated Liposomes

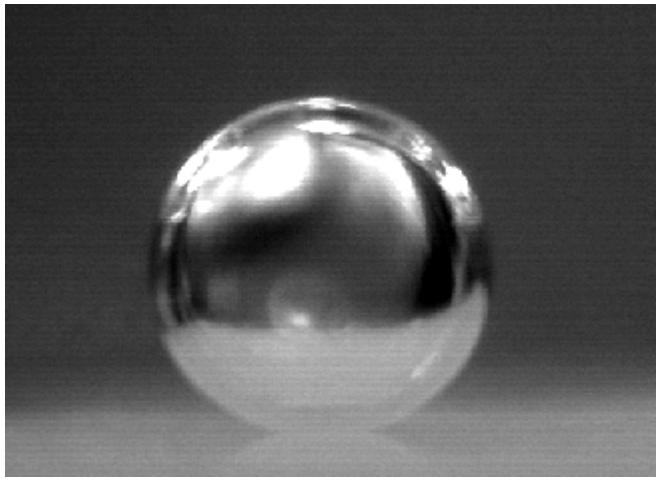


Decorated Liposomes



Nano-scale colloidal assemblies in foods

Dérick Rousseau, PhD
Department of Chemistry and Biology
Ryerson University
Toronto, Canada



USDA/NIFA-AFMNet meeting
Washington, DC
December 10-11, 2010

Driving force for nano-scale research

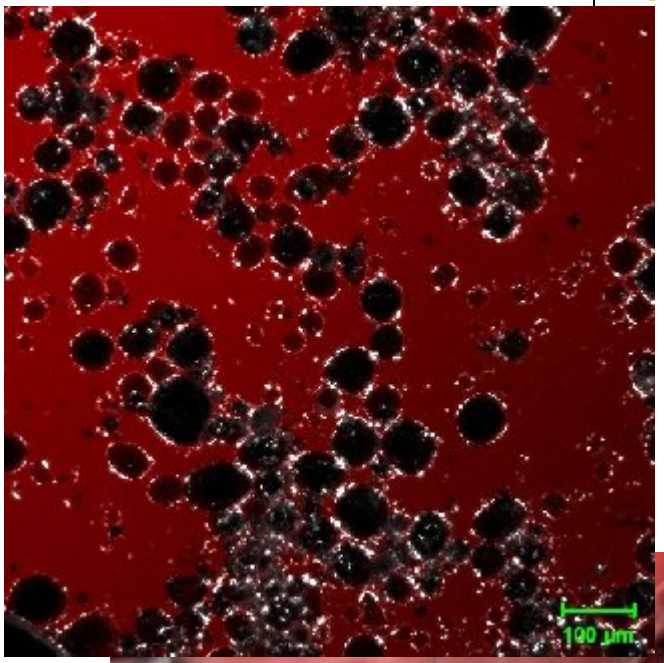
- Simple nano-scale matrices for food colloid stabilization and controlled release applications

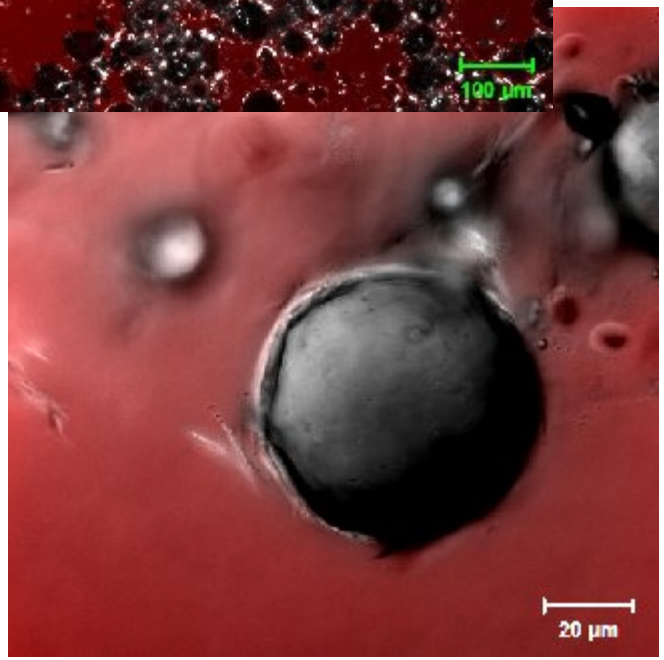
- Today: Brief overview of two projects:
  - Project 1: Solid lipid nanoparticles (SLNs) as Pickering O/W emulsion stabilizers
  - Project 2: SLNs as Pickering W/O emulsion stabilizers

- **Goal: Emulsion stabilization with SLNs**

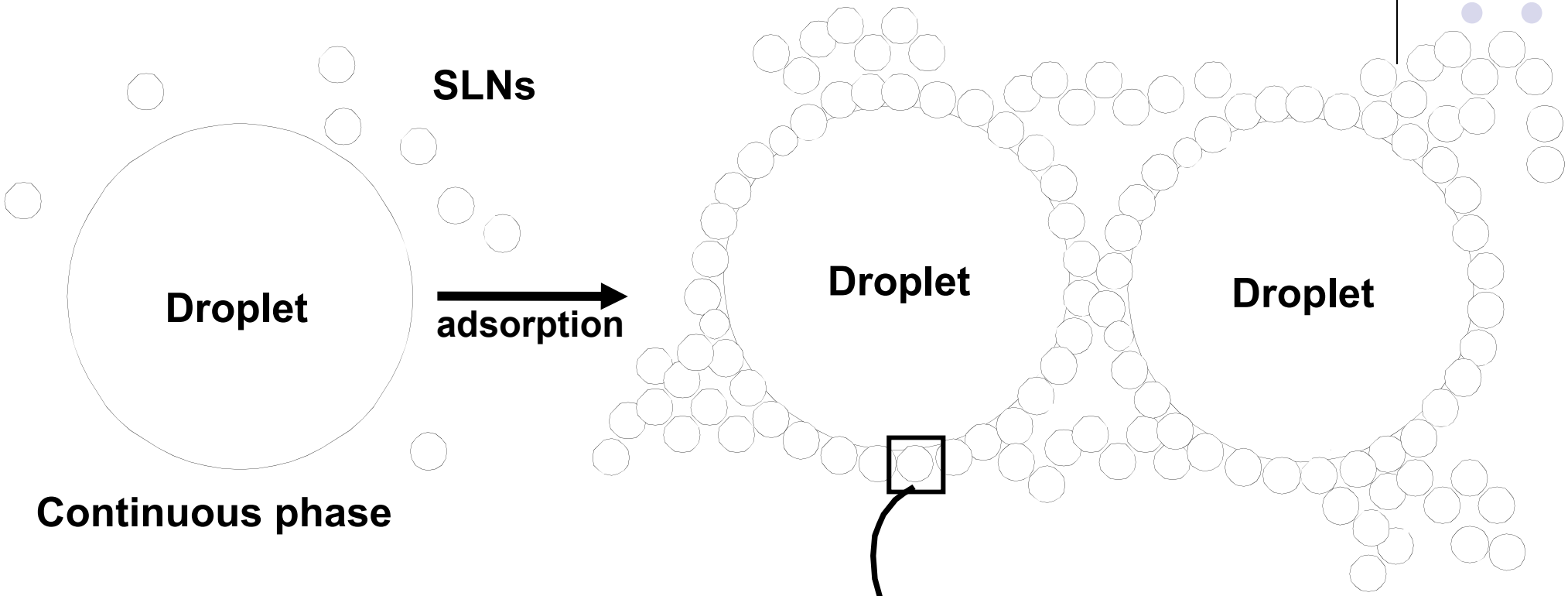
Pickering emulsions

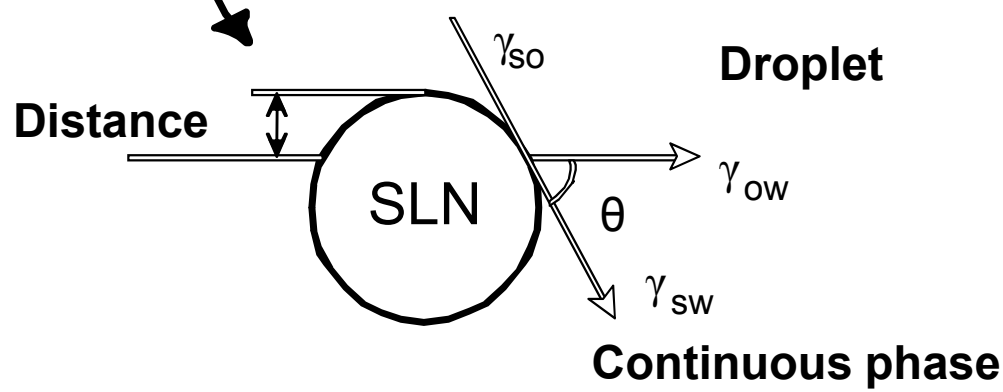
- Emulsions stabilized by µm-sized interfacial particles
- Particles form barrier limiting flocculation and coalescence
- Apps: Crude oil, cosmetics & wastewater treatment
- Nanoparticles as Pickering species: silver (5nm) & clay (35nm)
- **Lipid-based** nanoparticles have not been investigated as emulsion stabilizers





Pickering emulsion with SLNs





FACTORS:
Wettability
Morphology
Rheology
Location

SLNs as emulsion stabilizers

‘Nanoreactors’ to develop SLNs:

Hot (80°C) O/W microemulsion or nanoemulsions

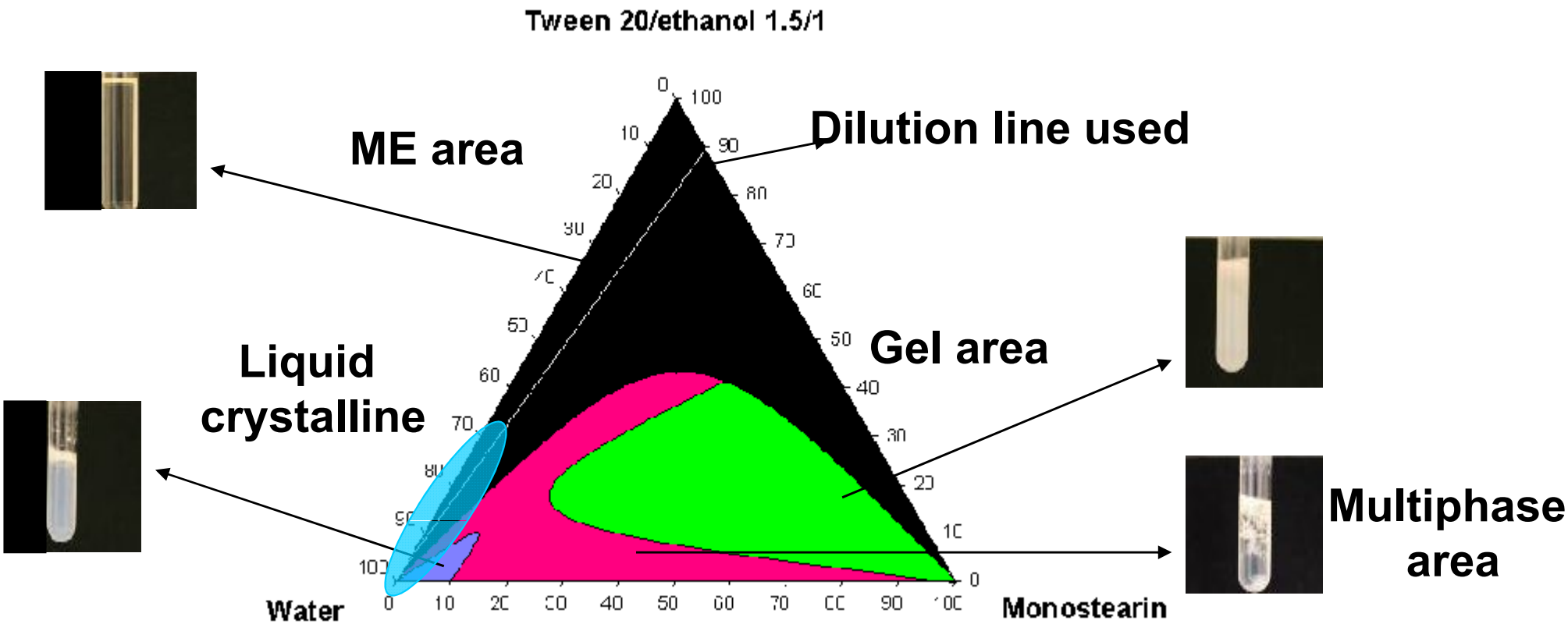
↓

Quench-cool hot micro/nano emulsions to ~4°C

↓

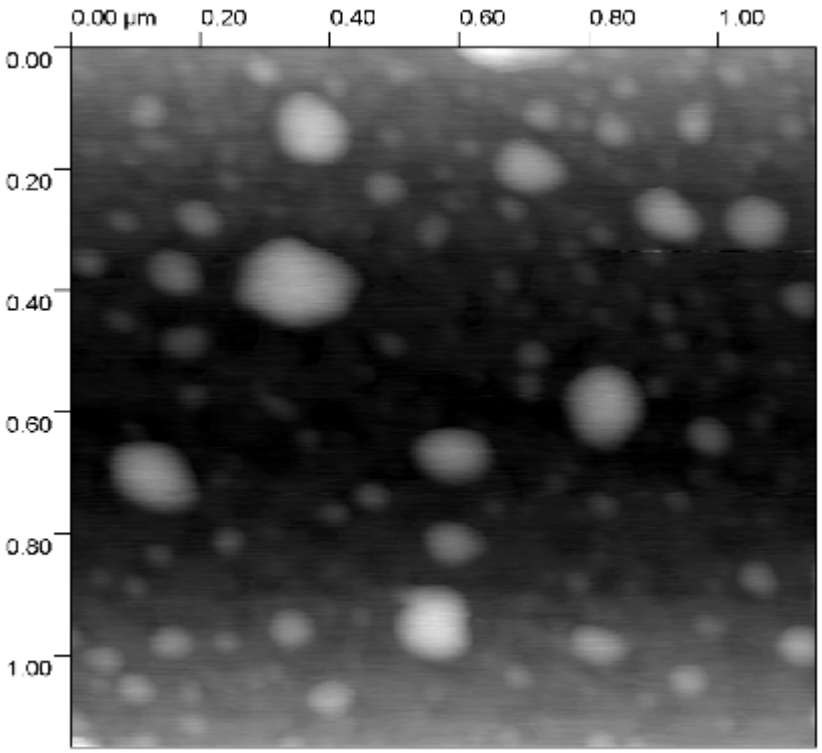
Prepare/characterize emulsions stabilized with SLNs

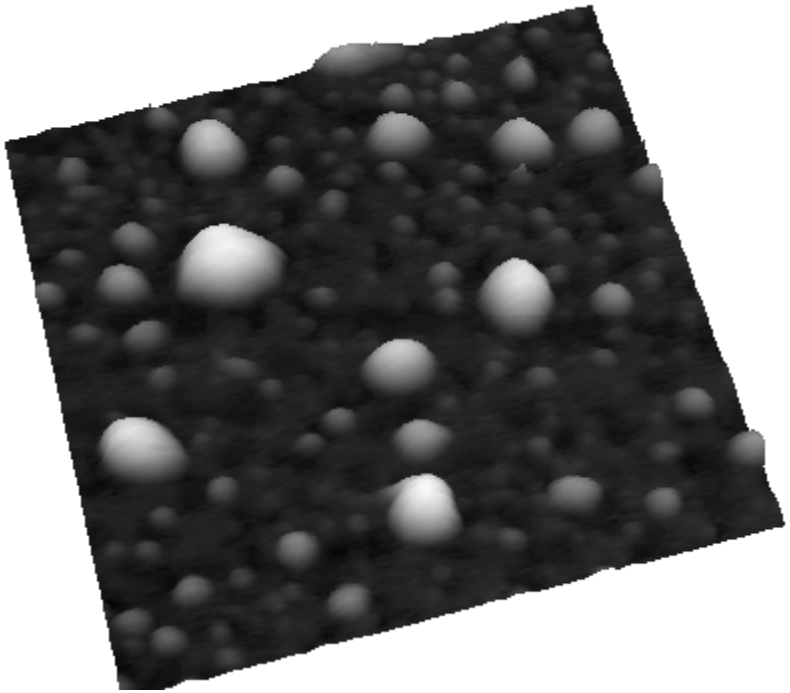
Project 1: Microemulsions as SLN ‘nanoreactors’



Composition: monostearin, water, Tween 20 and EtOH

SLN morphology via AFM



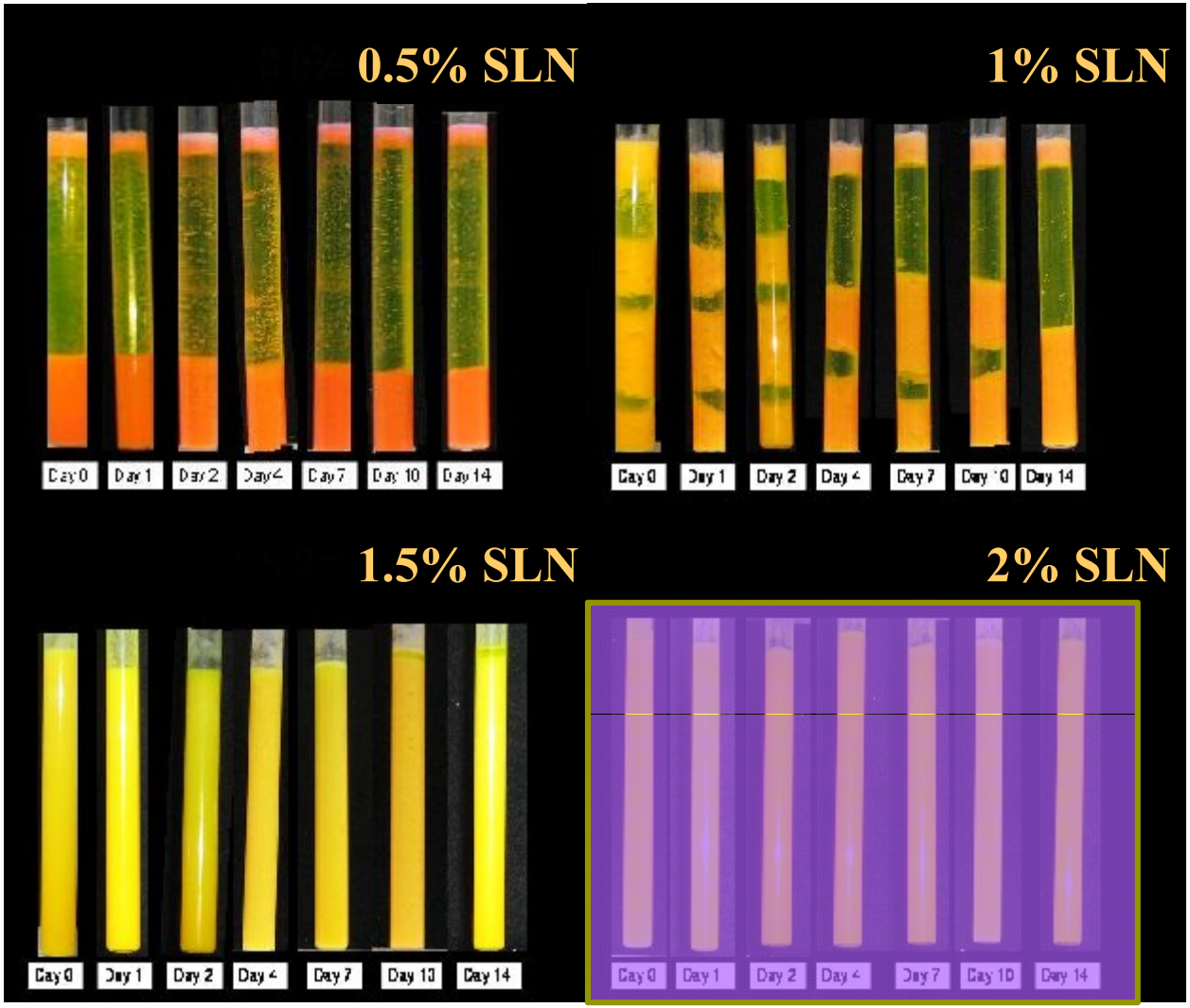


Particle size (nm) X ± SD	Polydispersity X ± SD	ζ-potential (mV) X ± SD
120.42 ± 18.30	0.15 ± 0.08	-40.0 ± 5.2

SLNs for emulsion stabilization

- Emulsion: homogenization of 20% water, oil and SLNs (0.5, 1, 1.5, 2% SLNs)
- Sedimentation
- Pulsed NMR for droplet size distribution
  - Mean diameter (d_{33}) and log normal drop size distribution (σ)
- Microstructure (combined fluorescence and polarized light microscopy)

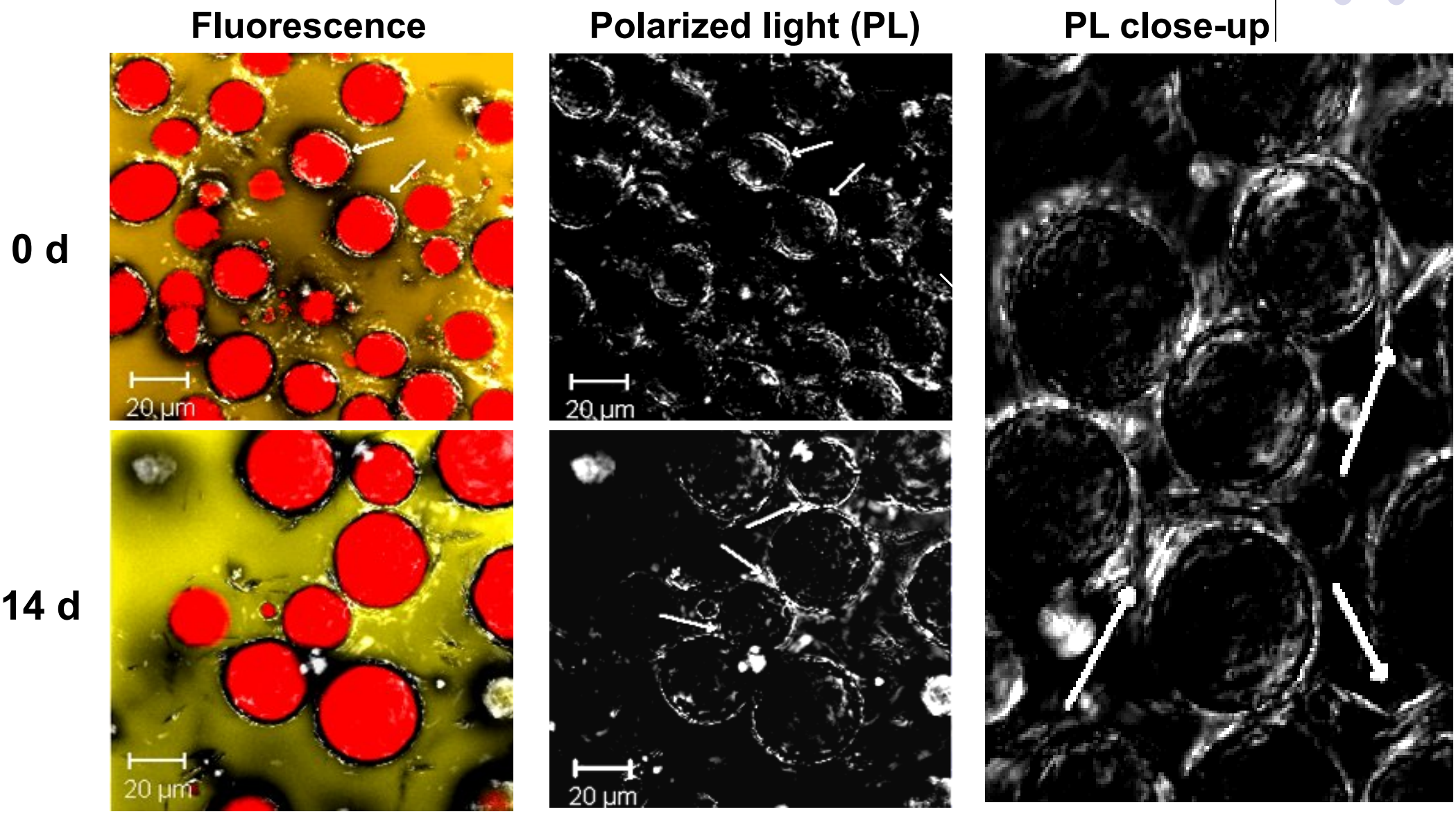
Sedimentation behaviour



- **0.5% SLN** - separated within minutes
- **1% SLN** - unstable within hours
- **1.5% SLN -**
  - better resistance to sedimentation
  - Some oil separation after 7 d
- **2% SLN** – no phase separation over 14 d

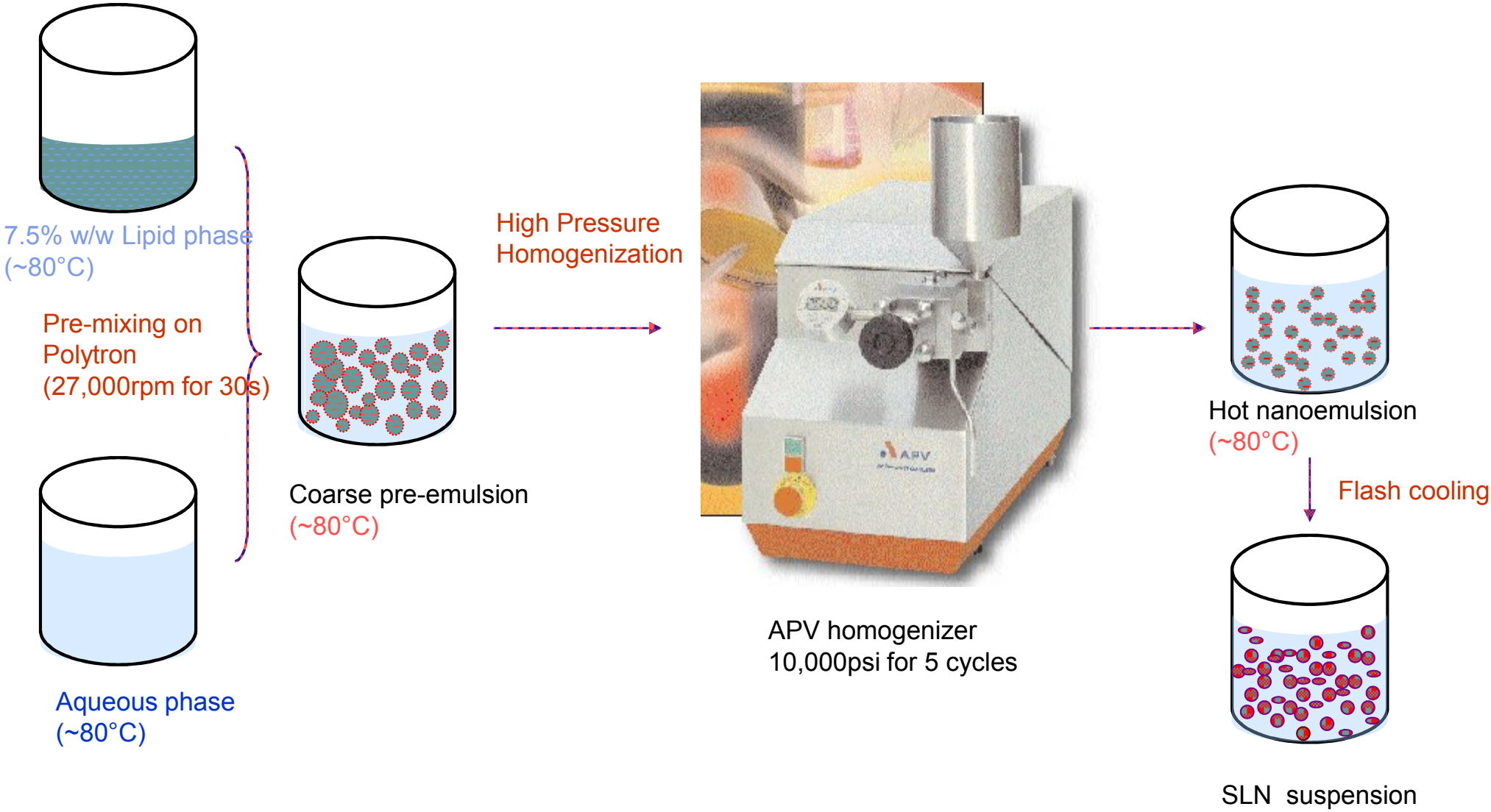
→No change in sedimentation behaviour over 2 wks
→Slight increase in drop size and polydispersity

Emulsion microstructure (2% SLN)

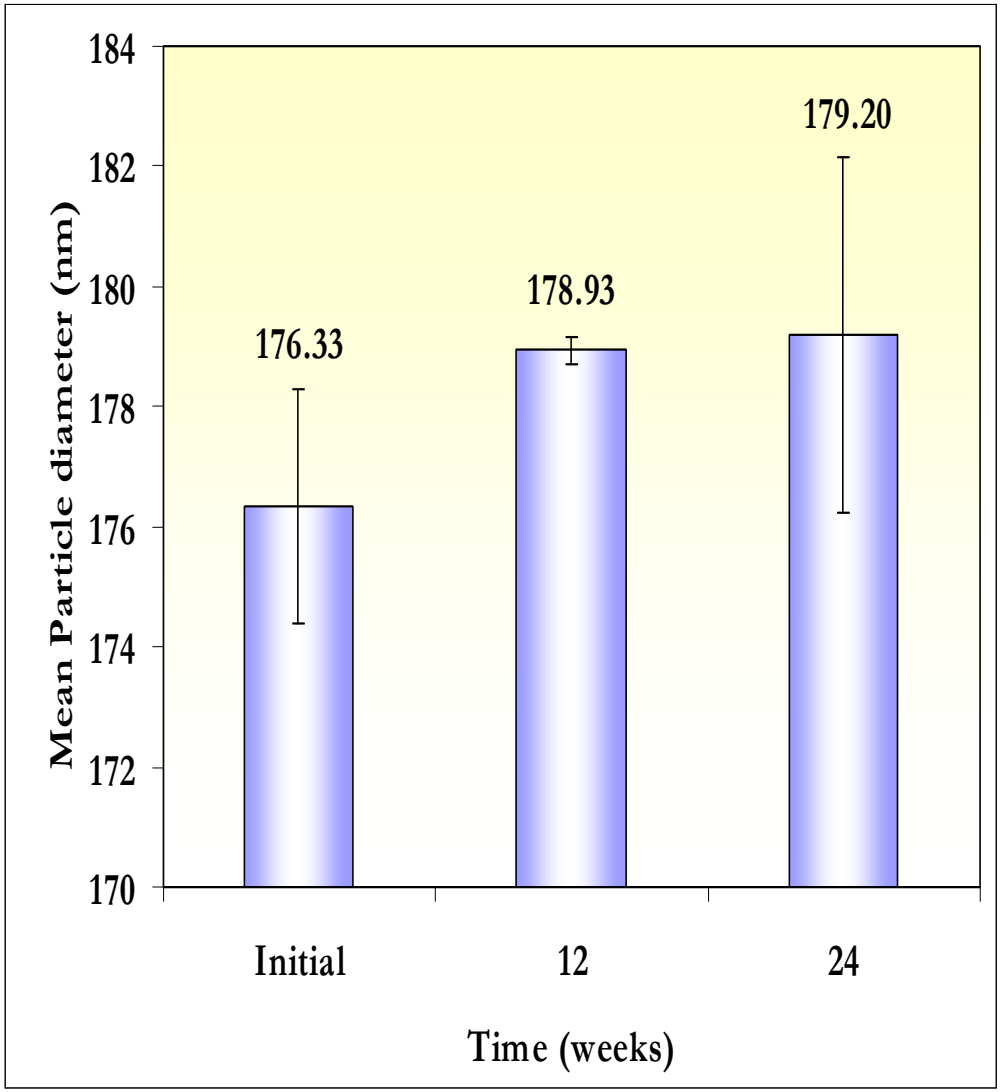


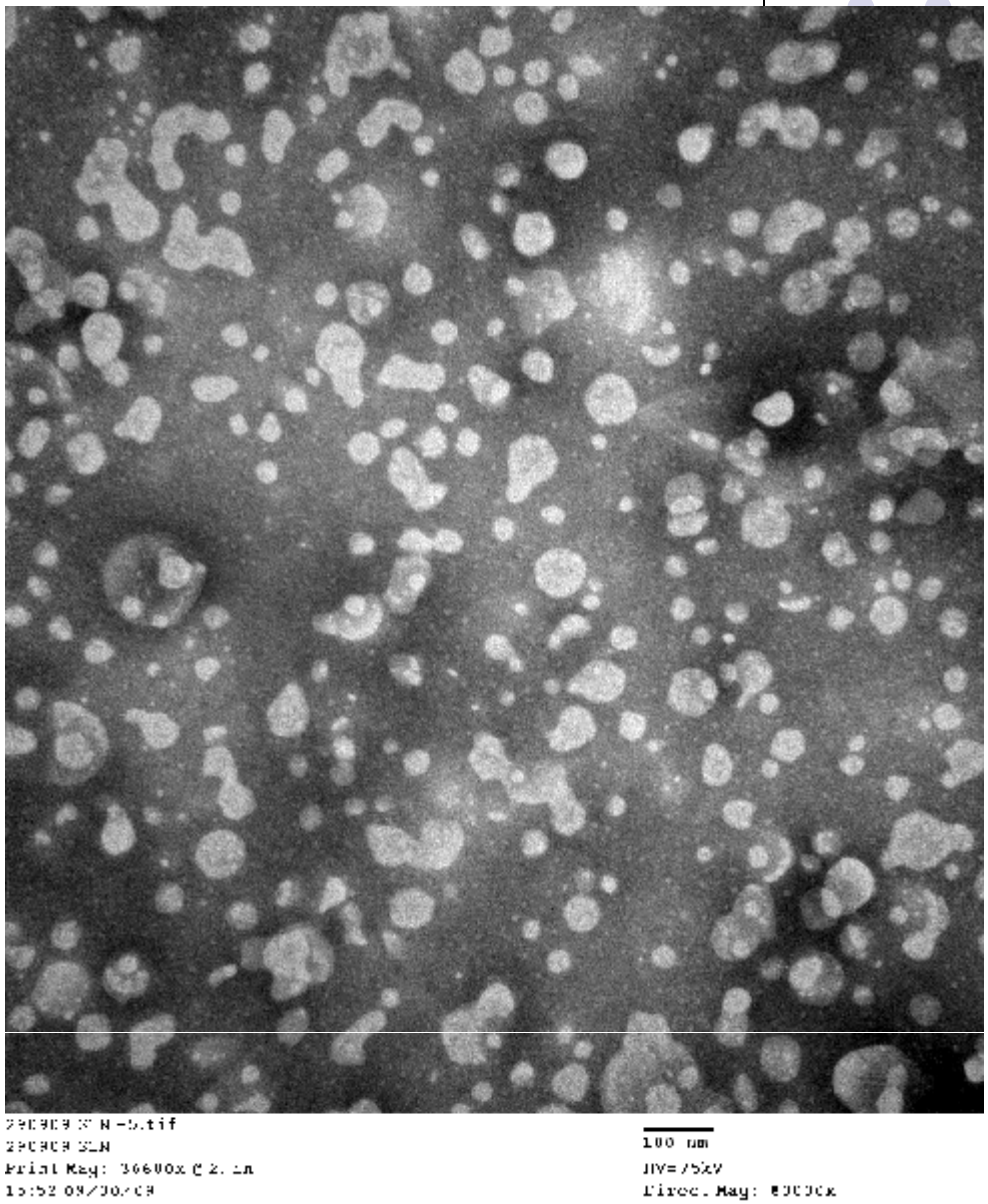
Project 2: Nanoemulsions for SLNs as Pickering stabilizers

Step 1: SLN suspension

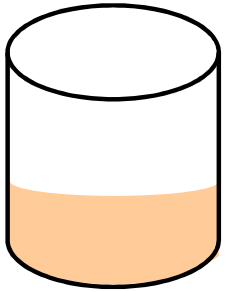


SLN particle size analysis



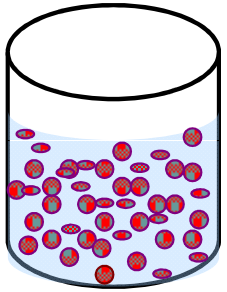


Preparation of oil-in-water emulsion stabilized with SLNs

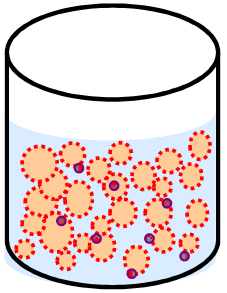


Oil phase 20%
(canola oil)
(~4-8°C)

Pre-mixing on Polytron
(27,000rpm for 30s)



Solid Lipid Nanoparticle
suspension 80% (~4-8°C)

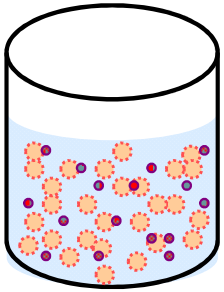


Coarse Pre-emulsion
(~4-8°C)

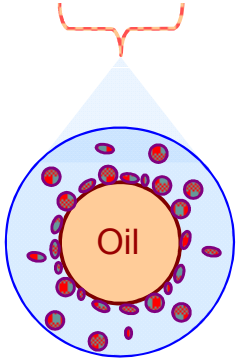
High Pressure
Homogenization

Ice bath

APV homogenizer
5000 psi for 5 cycles

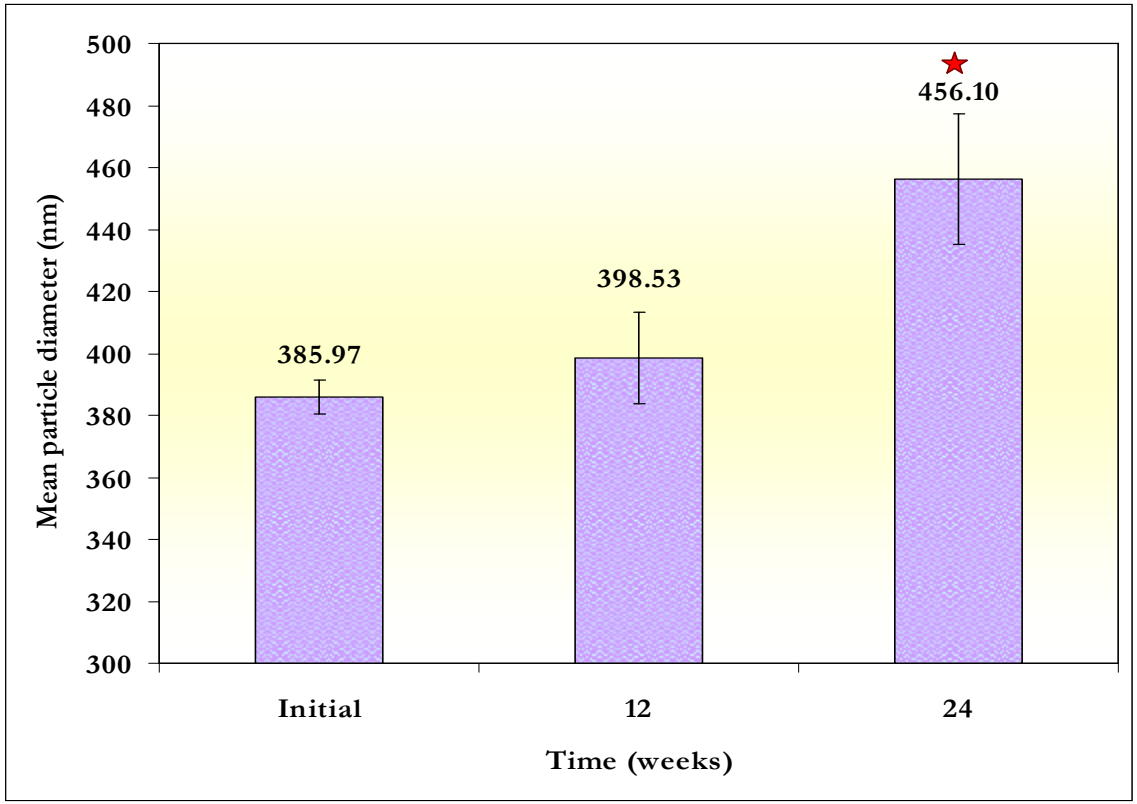


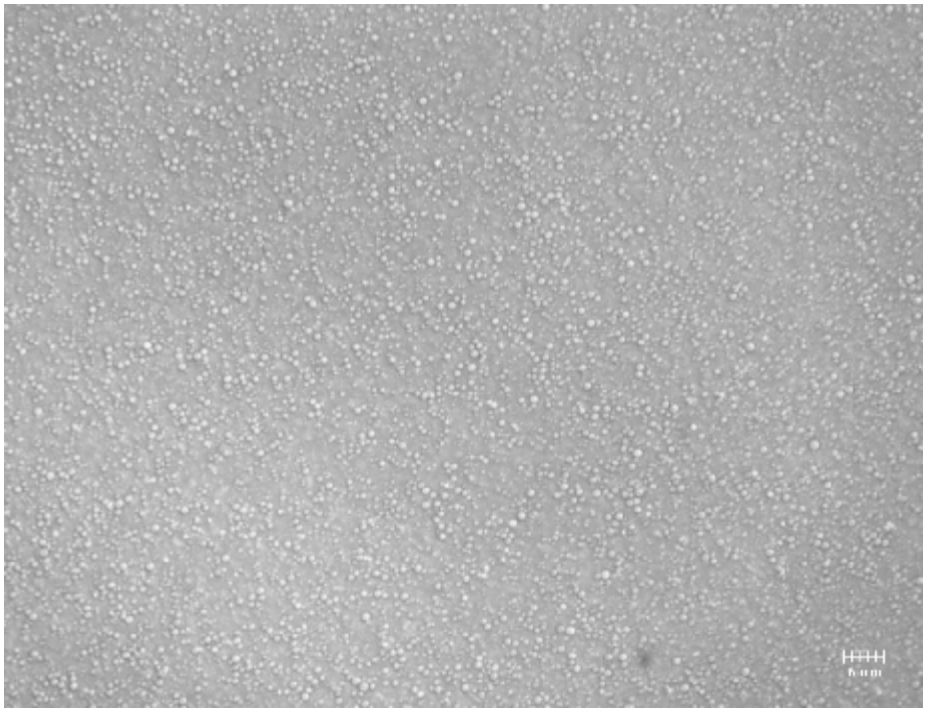
Oil-in-water (o/w)
emulsion



Oil droplets in o/w emulsion
solely stabilized by SLNs

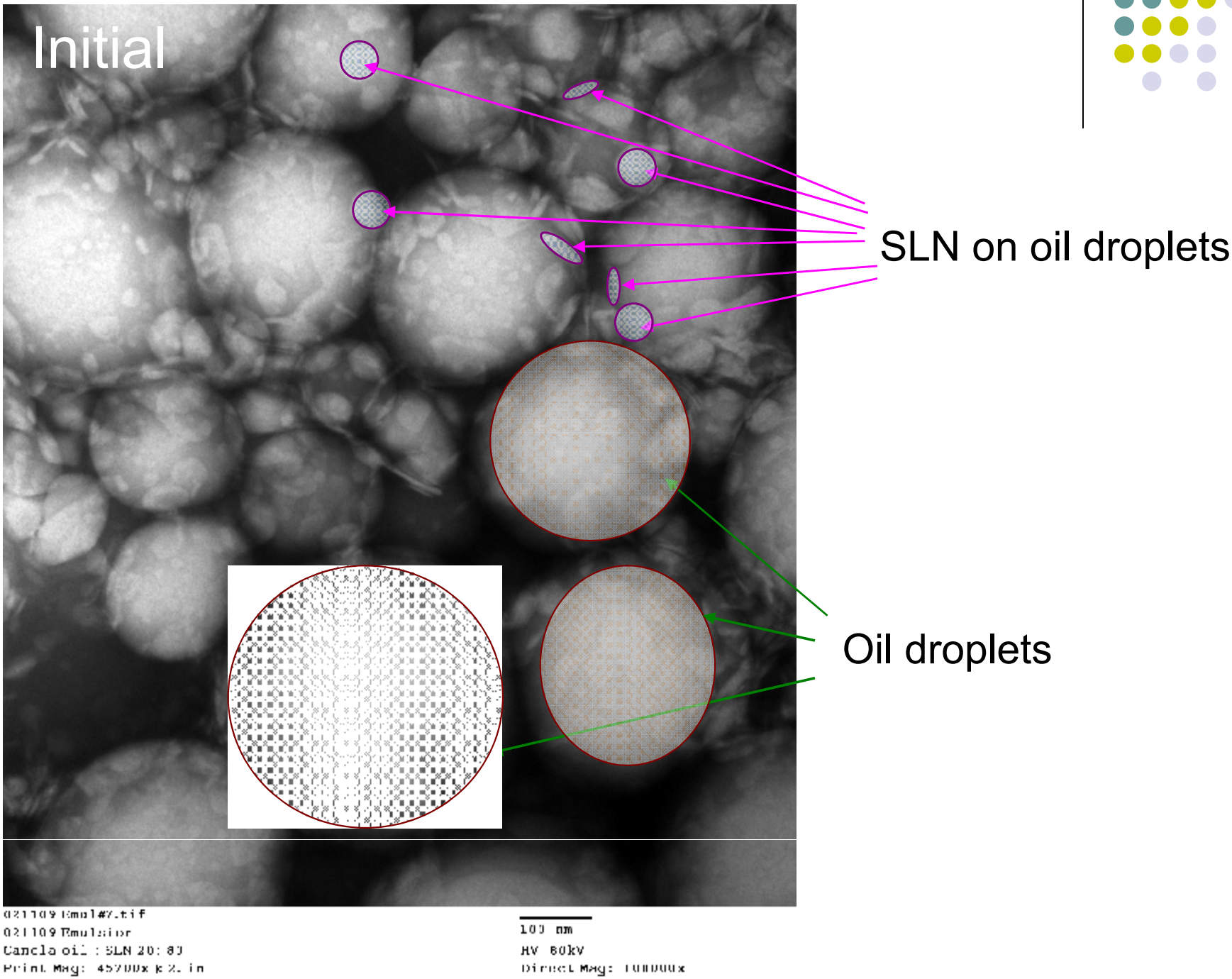
Evolution in emulsion droplet size with time



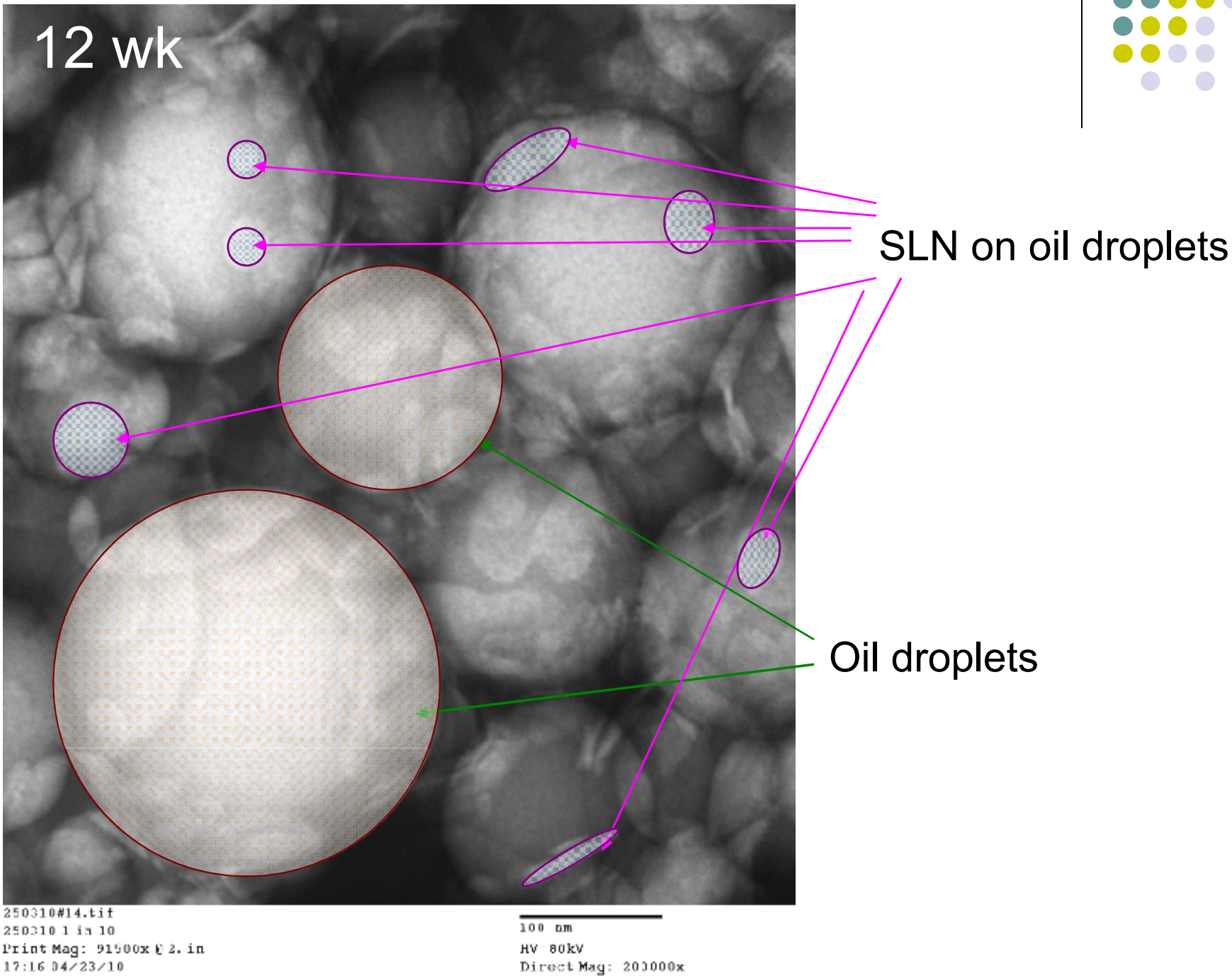


- Emulsions begin to slowly destabilize after 24 weeks
- Stabilization mechanism: Pickering SLNs

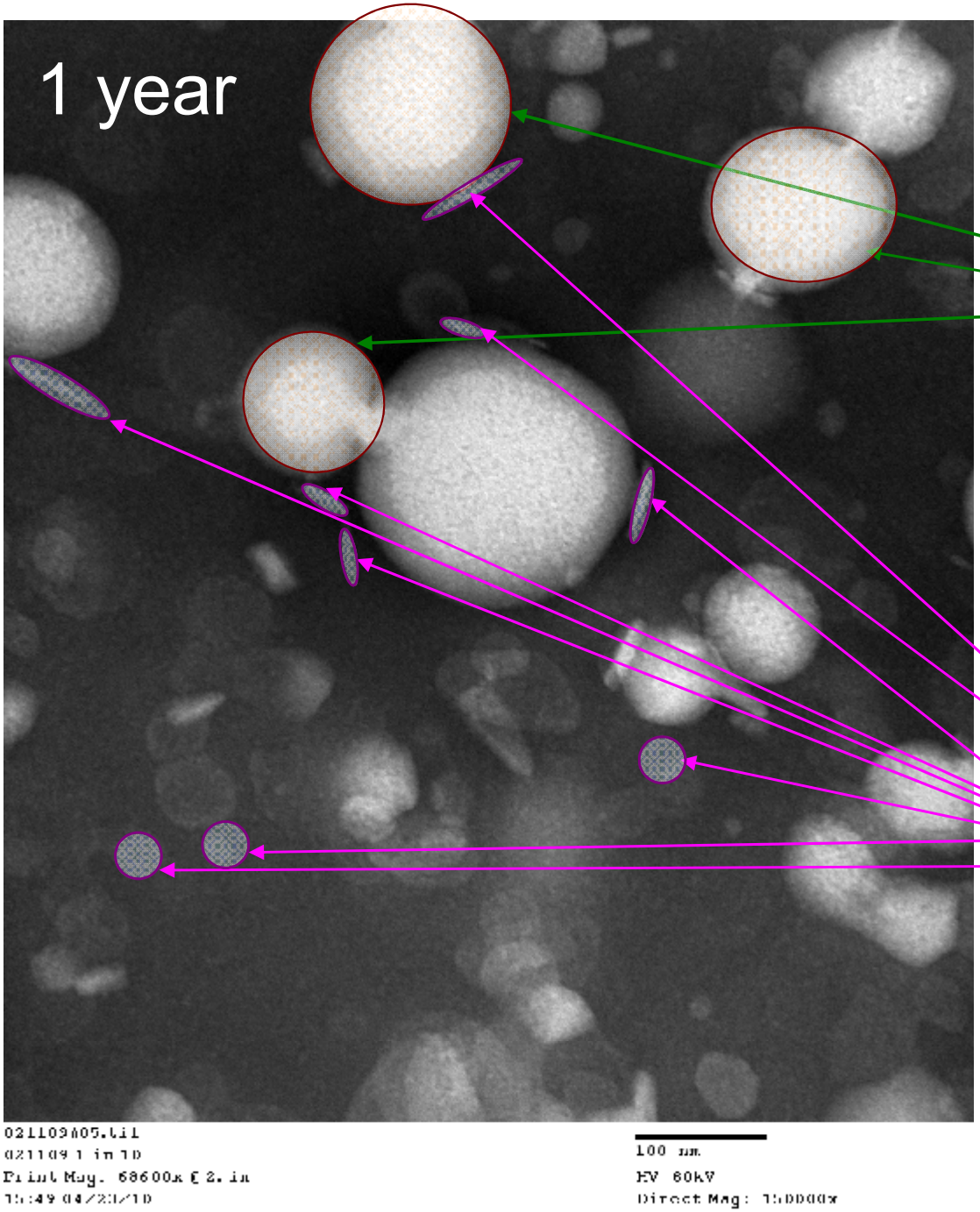
TEM of SLN-stabilized emulsions



TEM of SLN-stabilized emulsions



TEM of SLN-stabilized emulsions



Conclusions

- SLNs can be tailored as W/O or O/W emulsion stabilizers
- Kinetically-stable SLN-stabilized emulsions using food-grade components
- Surface-active lipids adsorbed at oil-water interface
- Advantage: Cold homogenization
  - preparation of emulsions containing thermo-labile components (*e.g.*, aromas, bioactive compounds).

Acknowledgements

- Students: Renuka Gupta and Elham Hazfi
- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Ryerson University
- Advanced Foods and Materials Network (AFMNet)

IMPROVED BIOACCESIBILITY AND BIOAVAILABILITY OF MODEL ANTIOXIDANT LIPOPHILIC VITAMIN

2ND YEAR REPORT

Cristina M. Sabliov[1] and Dorel Moldovan[2]
[1]Biological and Agricultural Engineering Department, LSU AgCenter
[2]Mechanical Engineering, LSU

December 2010

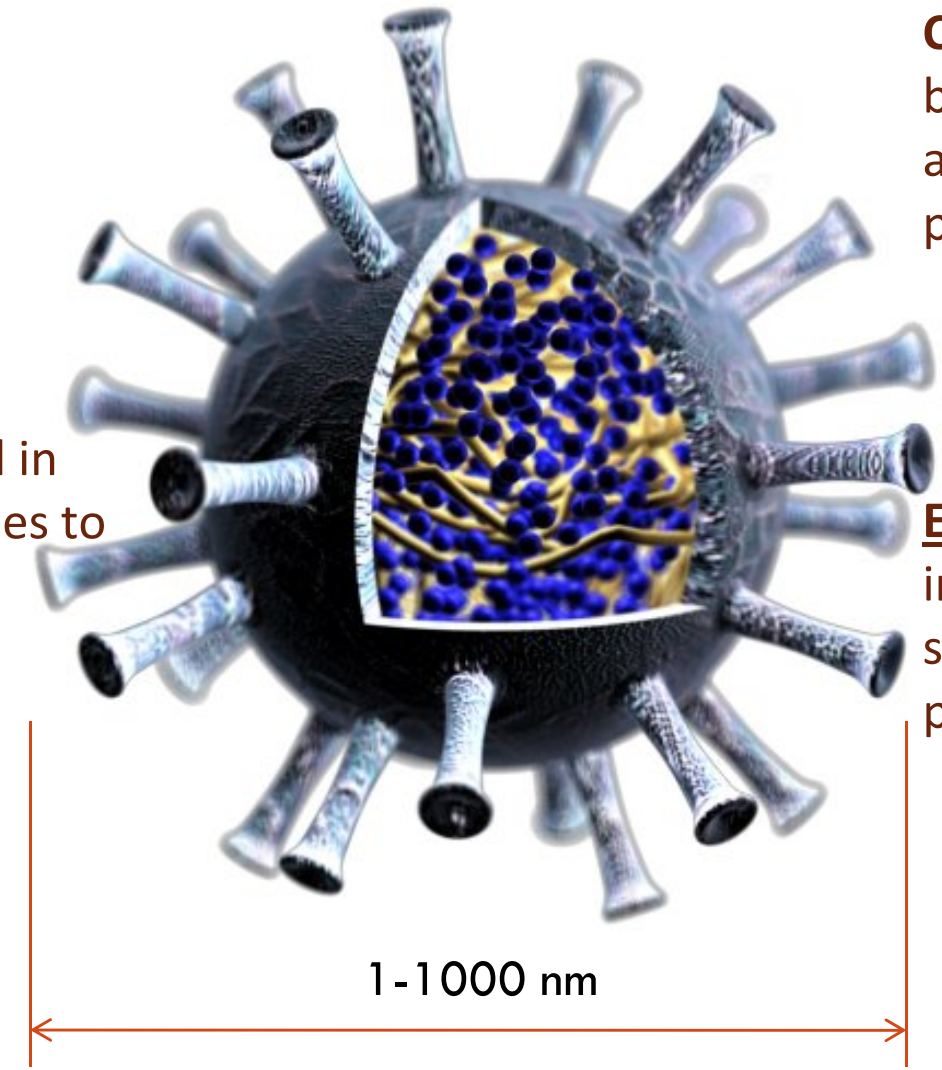
USDA-NRI 75.0 Nanoscale Science and Engineering for Agriculture and Food Systems

Polymeric nanoparticle

Protection: bioactive protected against degradation during storage and delivery

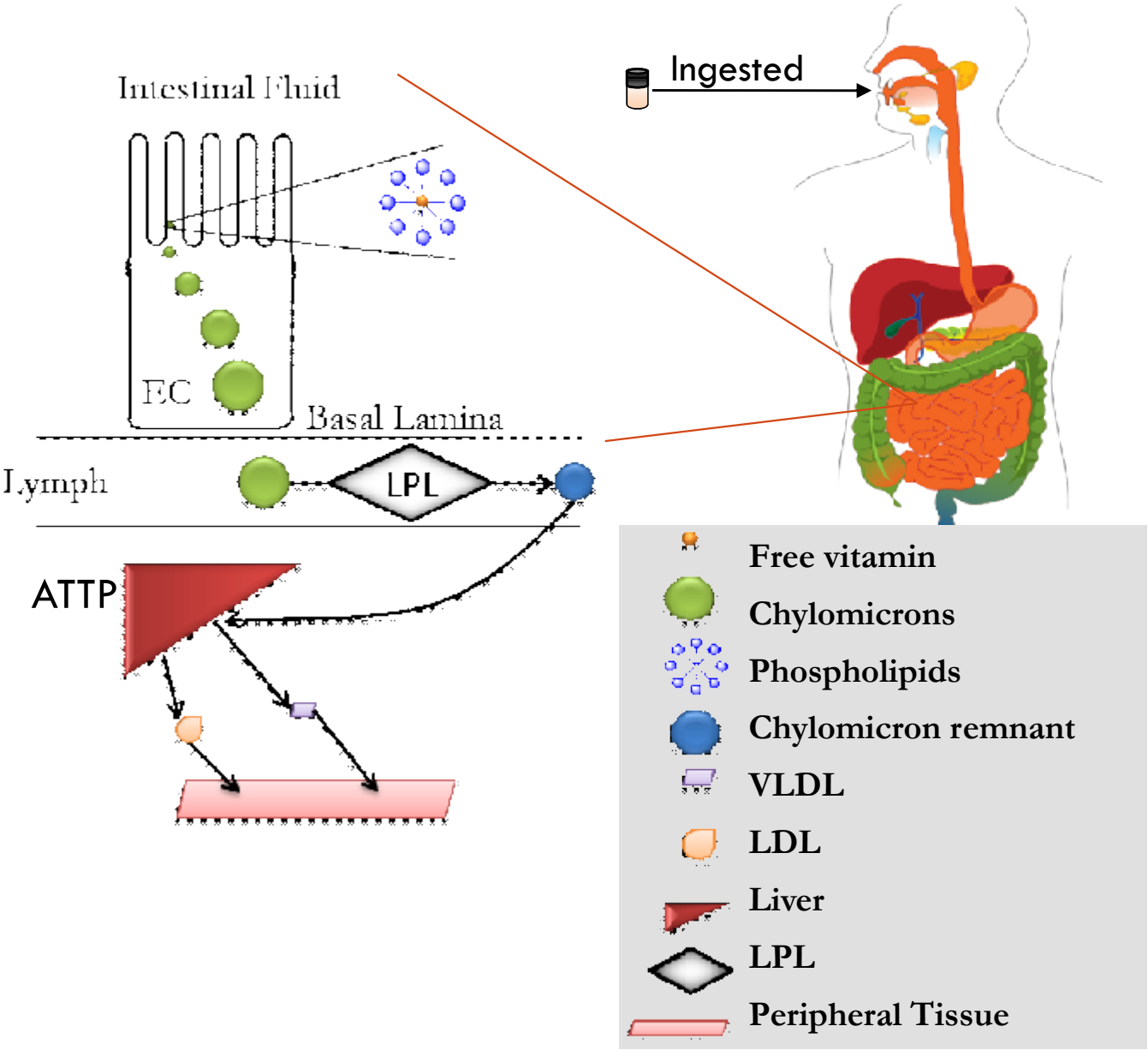
Controlled release: occurs by diffusion of bioactive and degradation of polymeric matrix

Targeting: ligands aid in targeting nanoparticles to the site of action



<u>Enhanced bioavailability</u>: improved due to nanoparticle size and controlled release profiles

Vitamin E transport



Bioavailability

Alpha-tocopherol

Intestinal uptake

Liver processing

Entrapment in a delivery vehicle

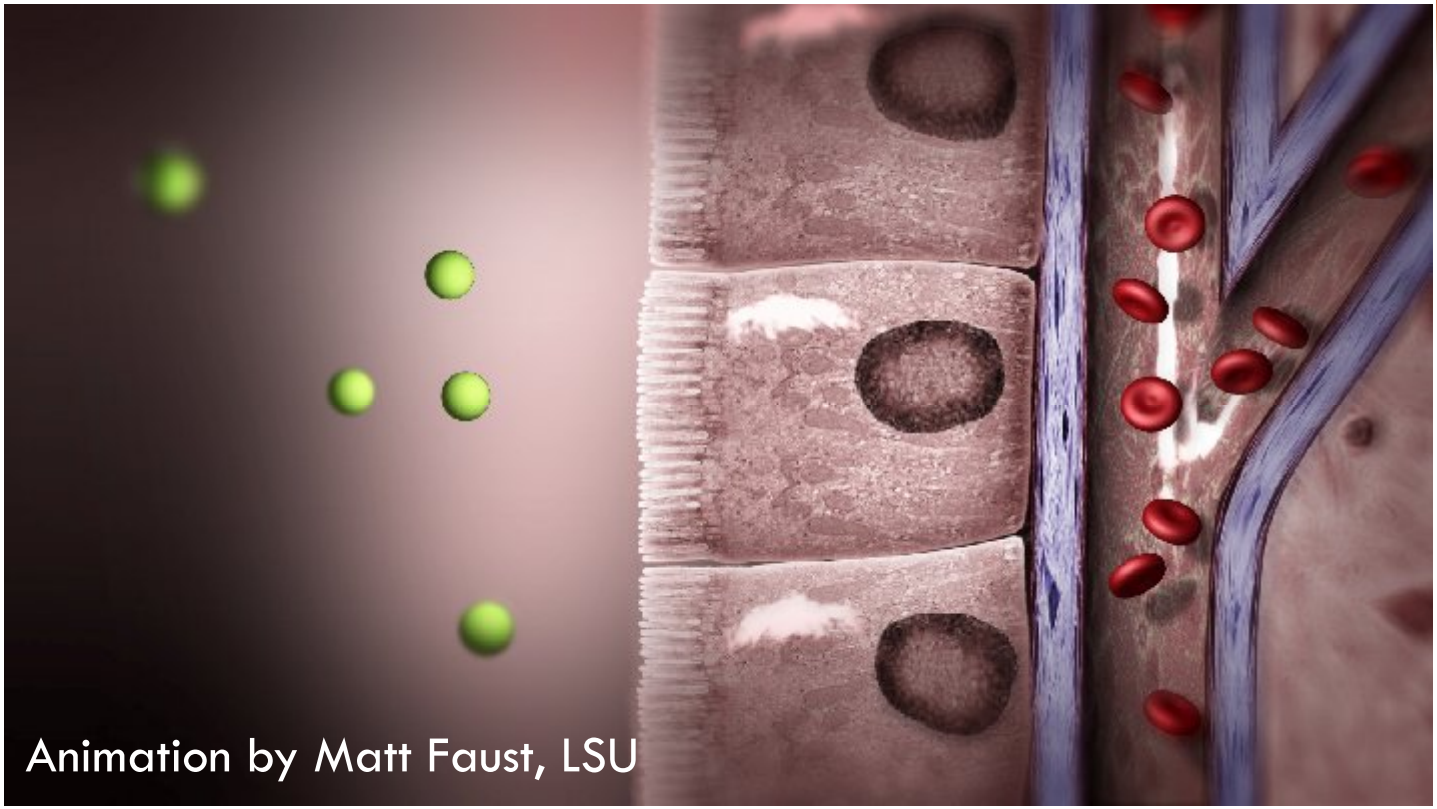
- solubility and transport in aqueous media
- controlled release of lipophilic substance from designed matrix
- enhanced uptake by enterocytes
- mucoadhesion increases gastrointestinal retention

Improved bioavailability

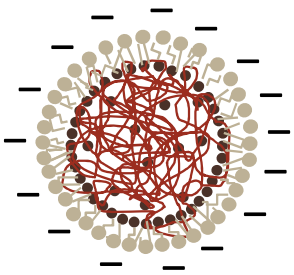
Objectives

- **Objective 1**: To synthesize and characterize Chitosan/PLGA nanoparticles with entrapped α-tocopherol (Chi/PLGA(αT)).
- **Objective 2**: To study the degradation and release profile of αT from the Chi/PLGA(αT) nanoparticles in simulated on-shelf, gastric, and intestinal environments
- **Objective 3**: To study the uptake of vitamin at intestinal site using atomistic molecular dynamics (MD) simulations and experimental studies.
- **Objective 4**: To study the mucoadhesion of the Chi/PLGA(αT) NPs, and the *in vivo* uptake of the vitamin released from Chi/PLGA(αT) using animal models.

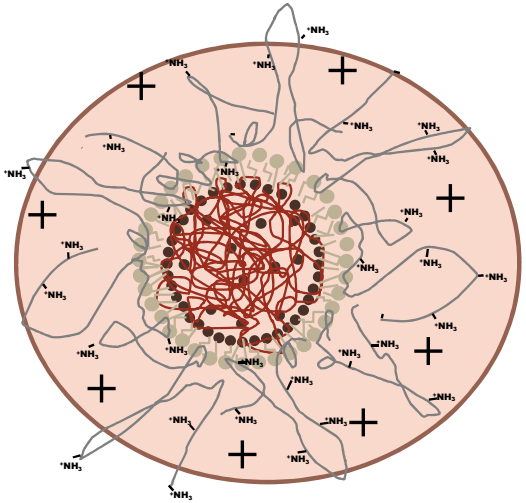
NP intestinal uptake



Delivery systems

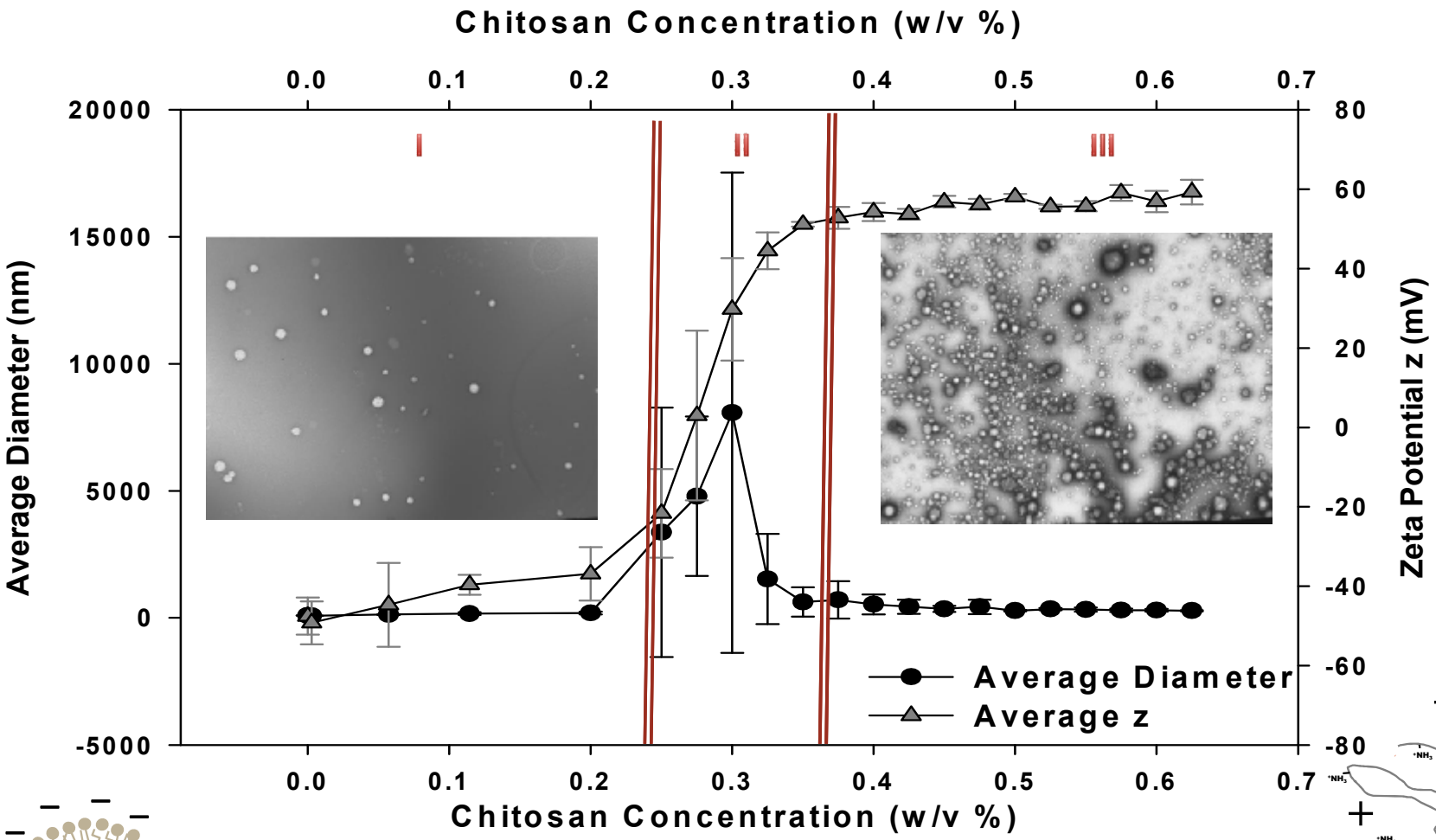


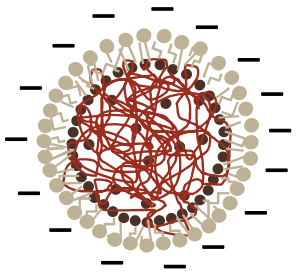
PLGA

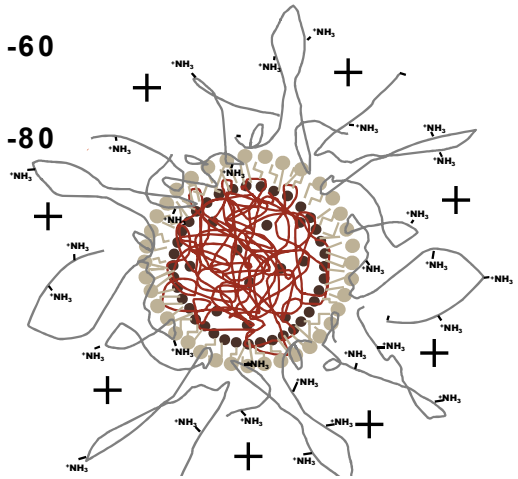


Chi/PLGA

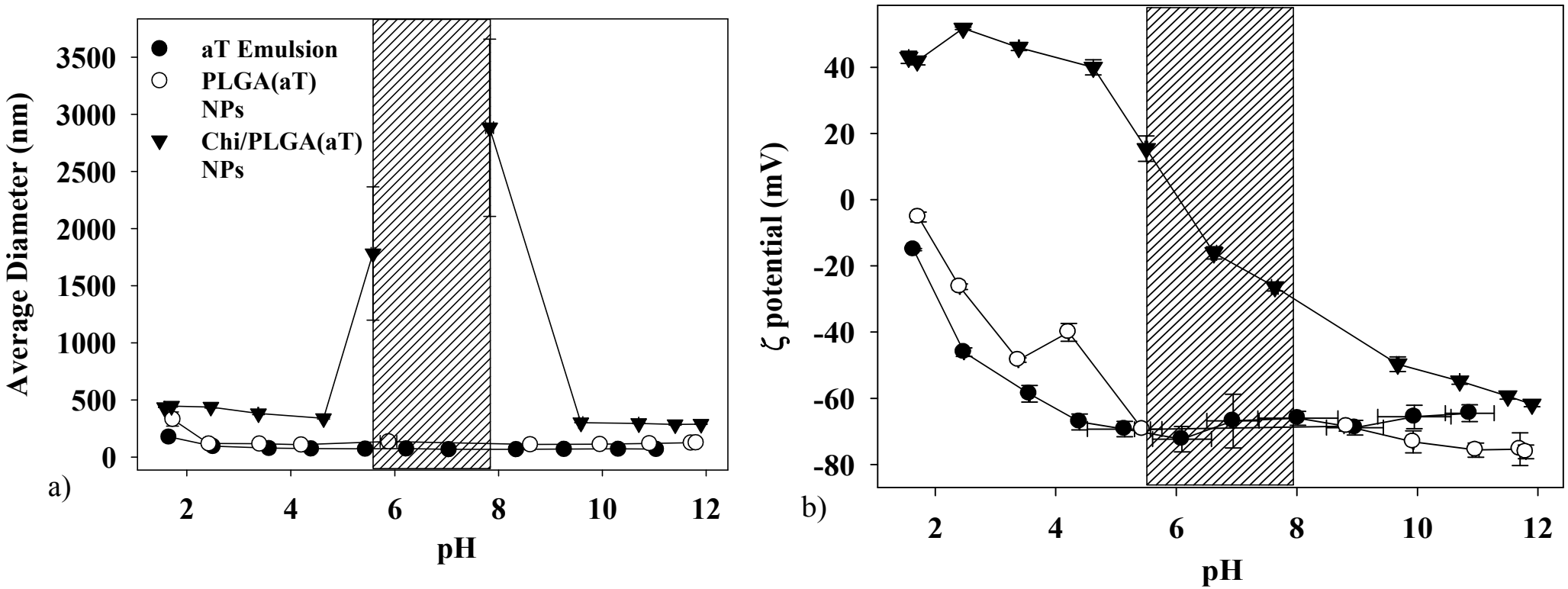
Chitosan and Chitosan/PLGA NPs







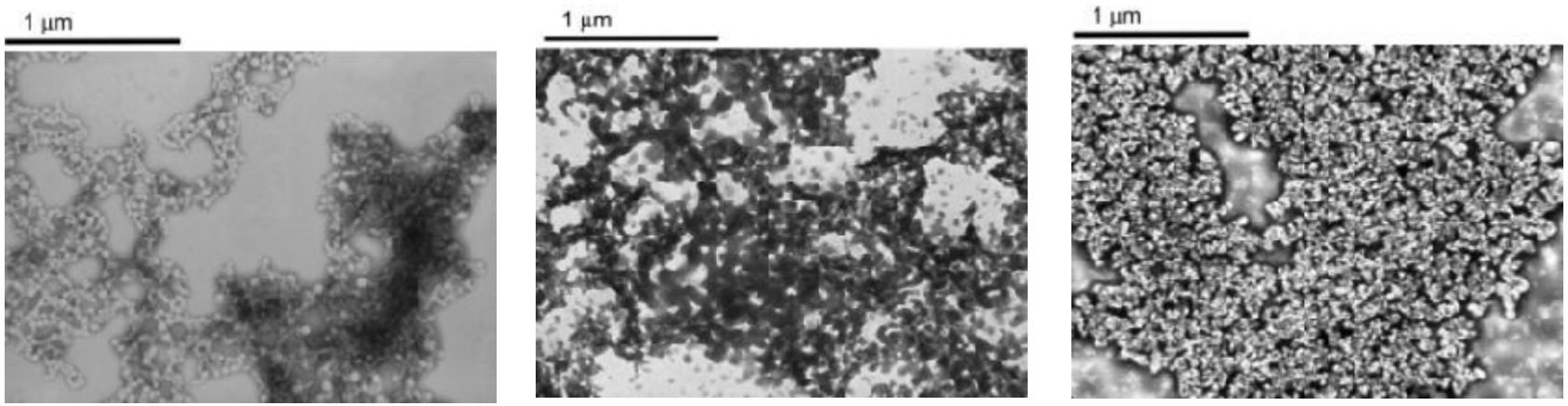
pH stability of particles



- PLGA particles were negatively charged and approached zero below pH 2
- Chi/PLGA particles approached zero between pHs 5-8

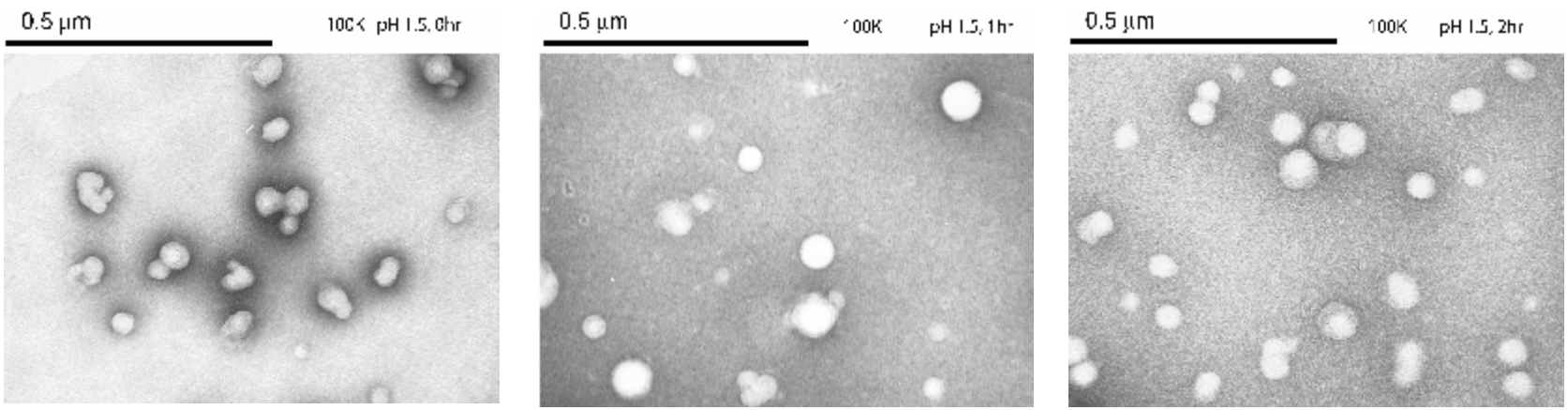
Particle morphology under gastric pHs

PLGA
33K
pH 1.5



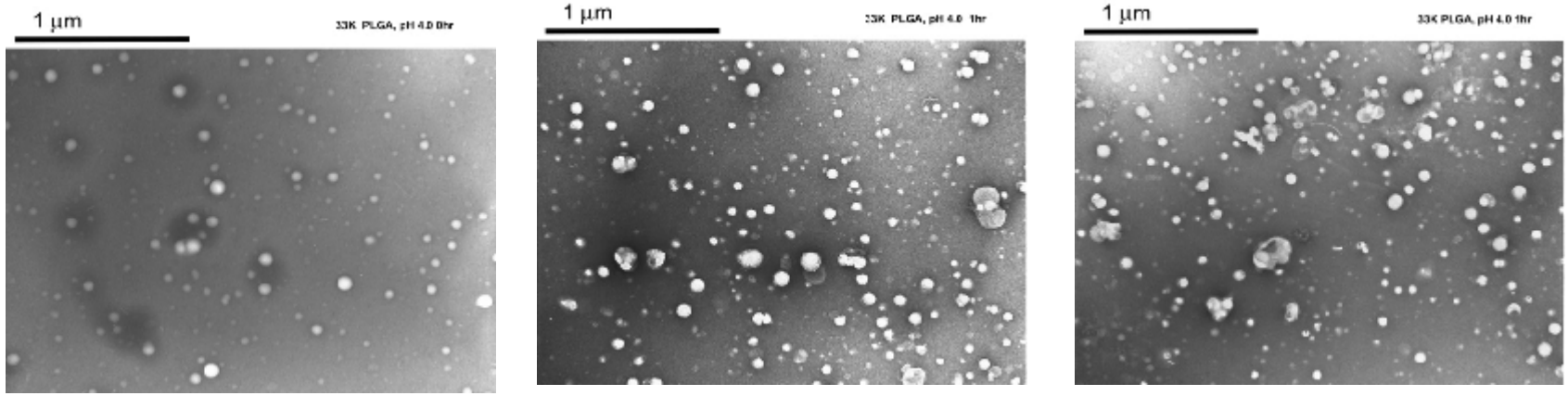
0 hrs → **1 hrs** → **2 hrs**

Chi/PLGA
100K
pH 1.5



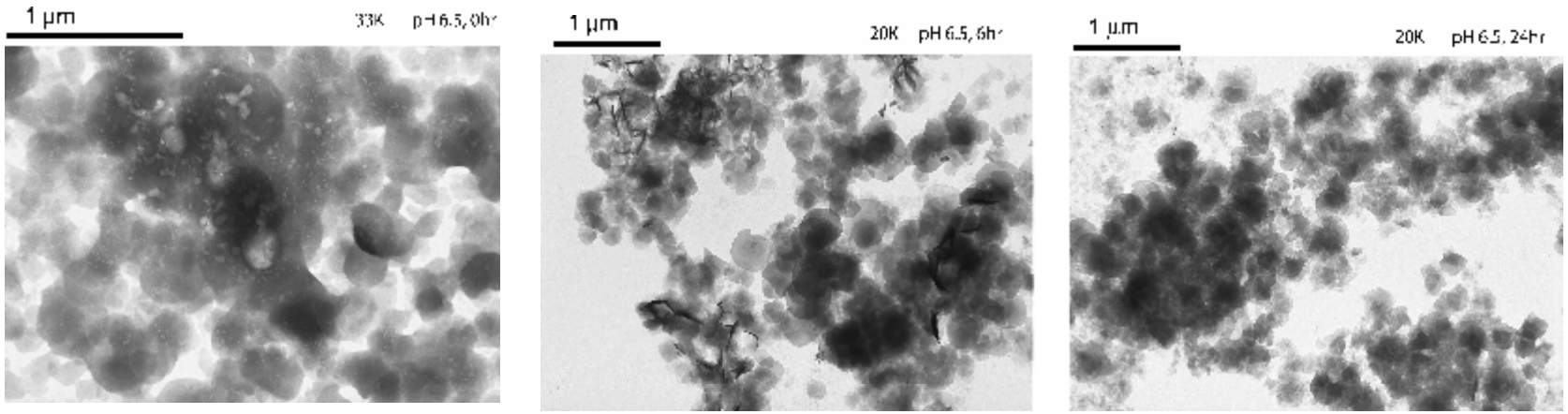
Particle morphology under intestinal pHs

PLGA
33K
pH 6.5

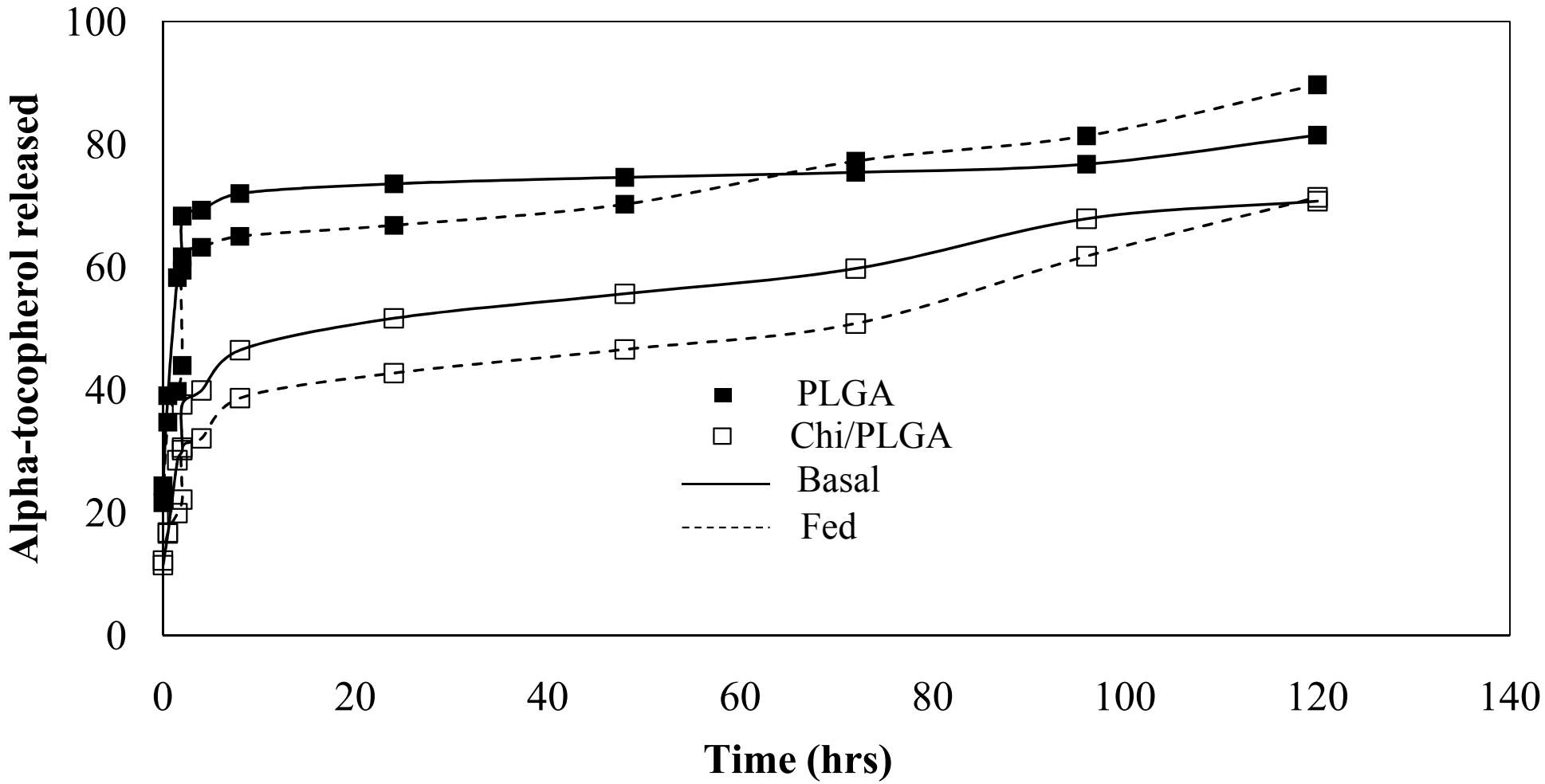


0 hrs → **6 hrs** → **24 hrs**

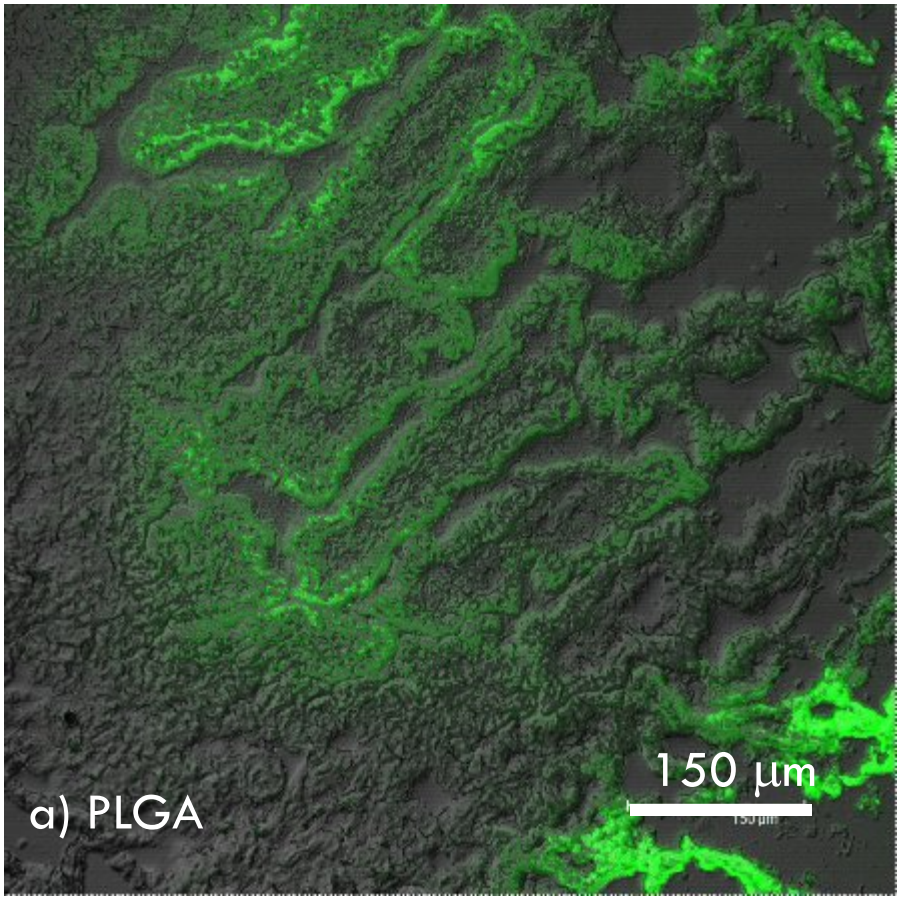
Chi/PLGA
20 K & 33K
pH 6.5

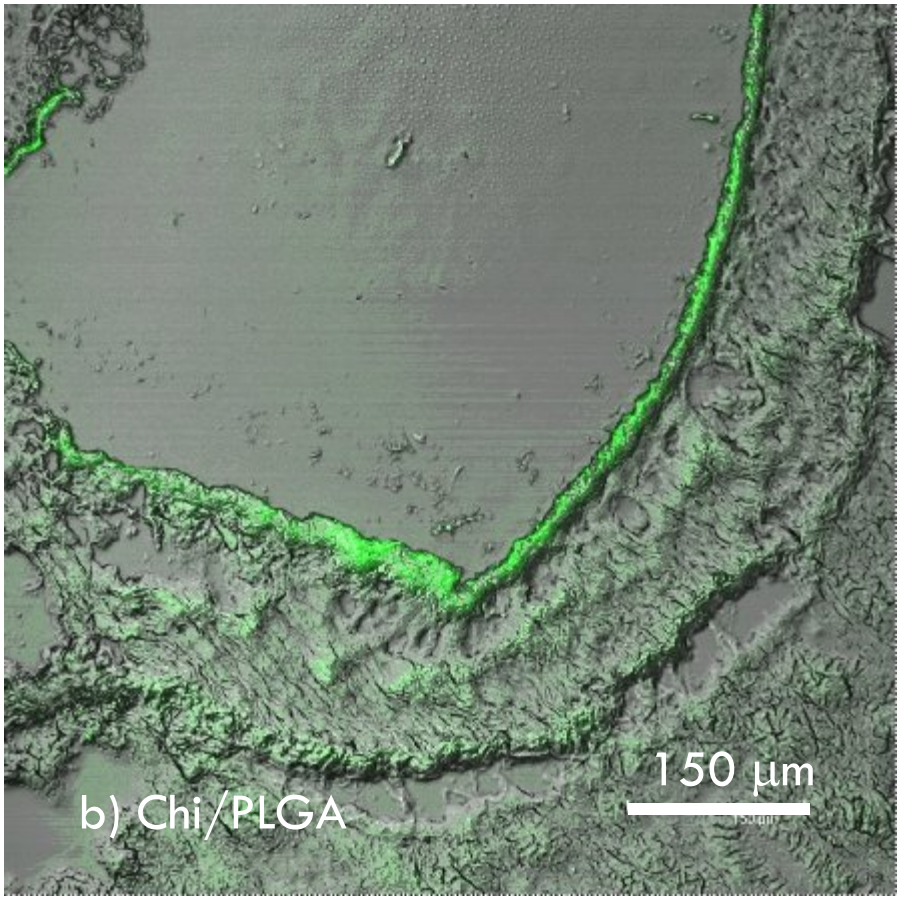


Gastrointestinal release of alpha-tocopherol



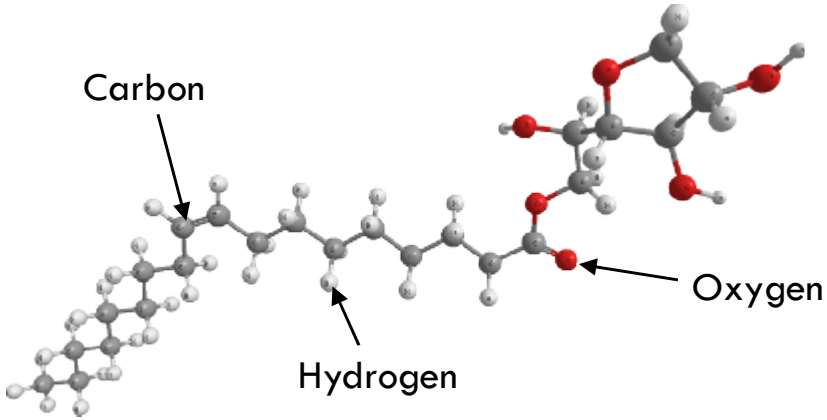
Ex-vivo nanoparticle uptake studies





Molecular dynamics simulation study of self-assembly of span 80 into micelles and their interaction with vitamin E

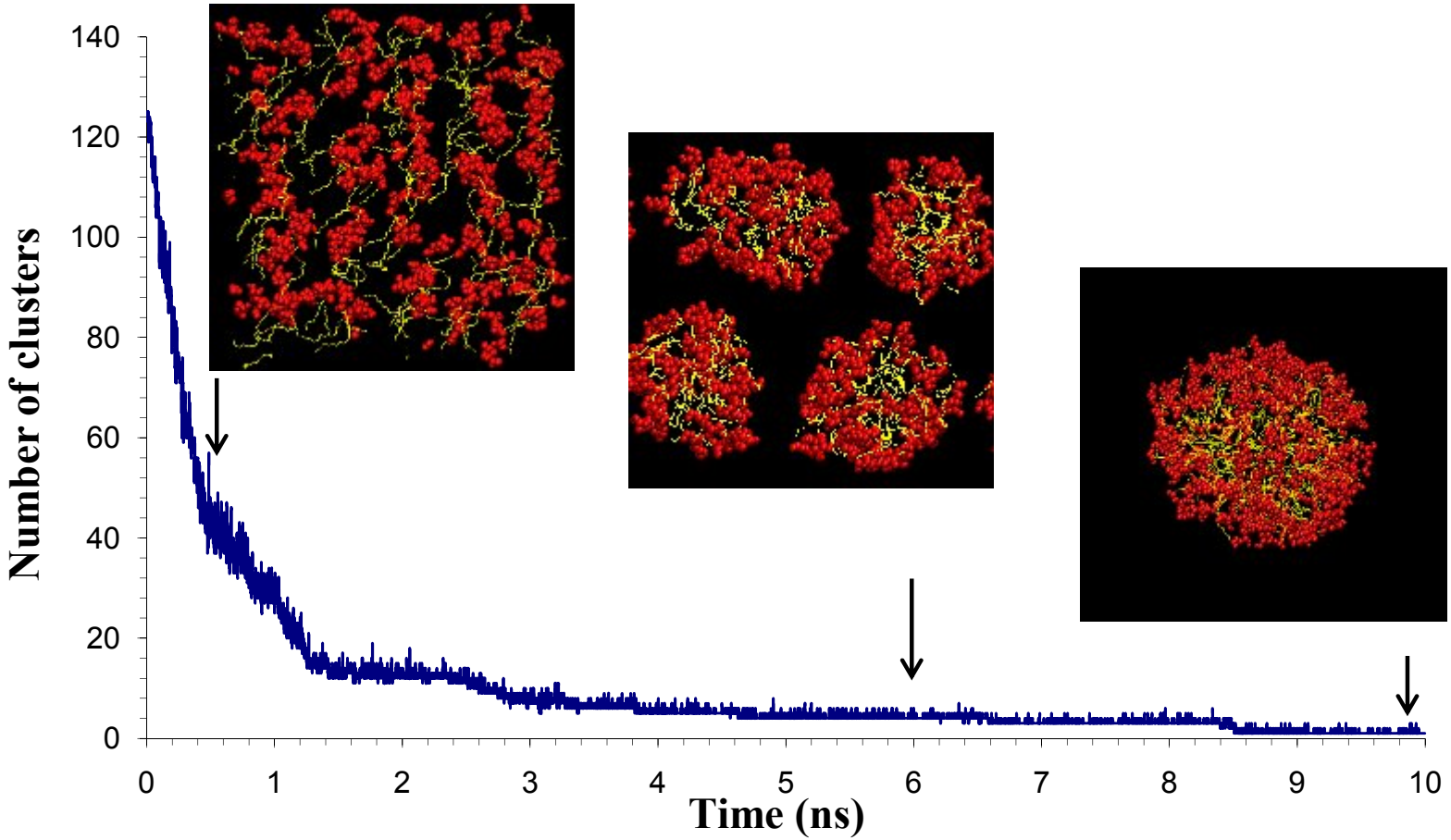
Simulation Methodology



Span80 molecule

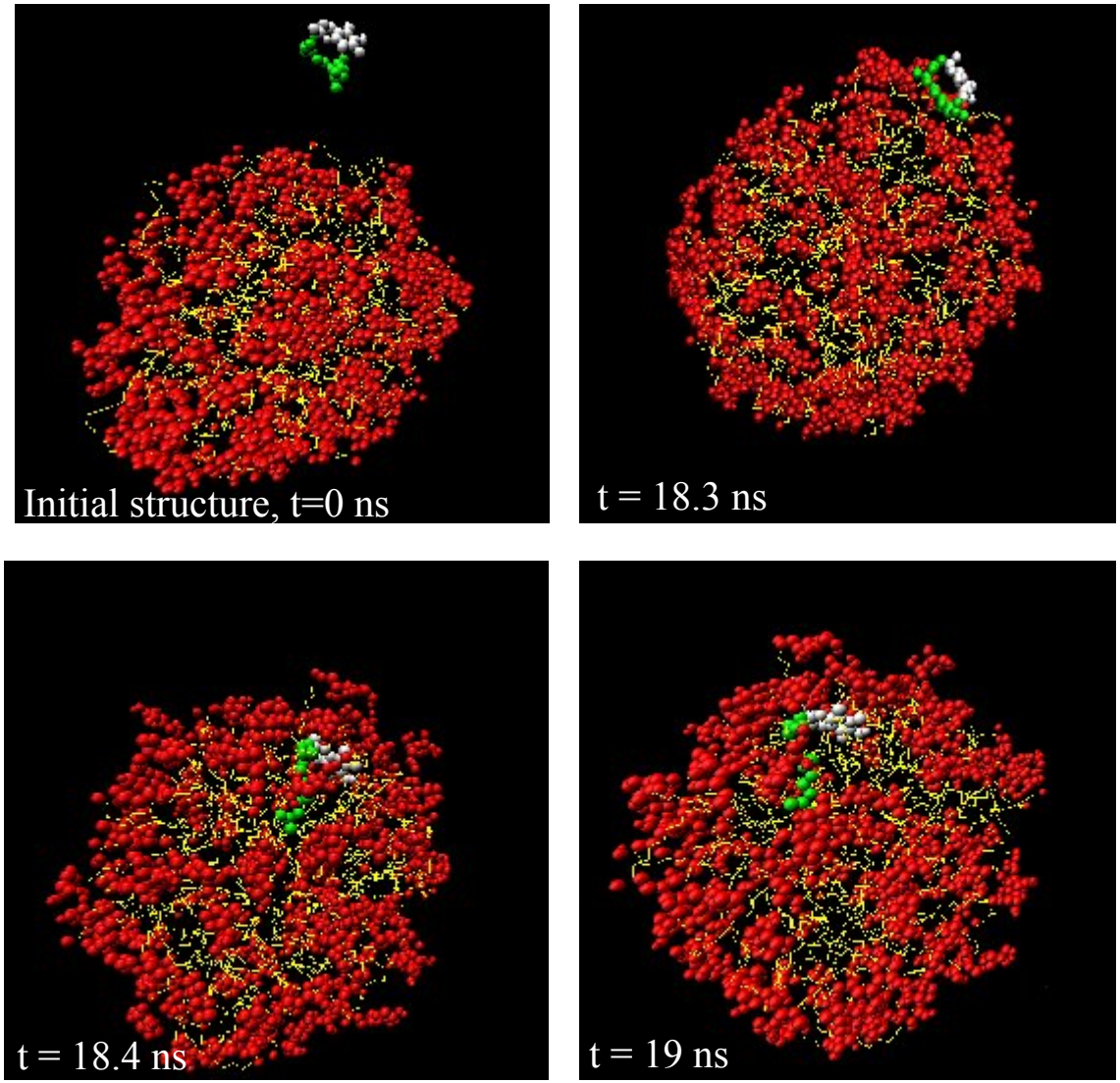
- Molecular dynamics simulations were performed with GROMACS 4.0 simulation package[1] on LONI Supercomputers at LSU.
- The force field parameters for bonded and non-bonded interactions for Span80 and alpha-tocopherol were generated by PRODRG2.5 server. The SPC model was used for water.
- The simulations were performed at constant pressure (1atm) using semi-isentropic pressure coupling and at constant temperature (T=300K).
- The simulation system consists of 125 Span80 molecules and 24,000 water molecules.

Time evolution of self-assembly of 125 Span 80 molecules into a single micelle



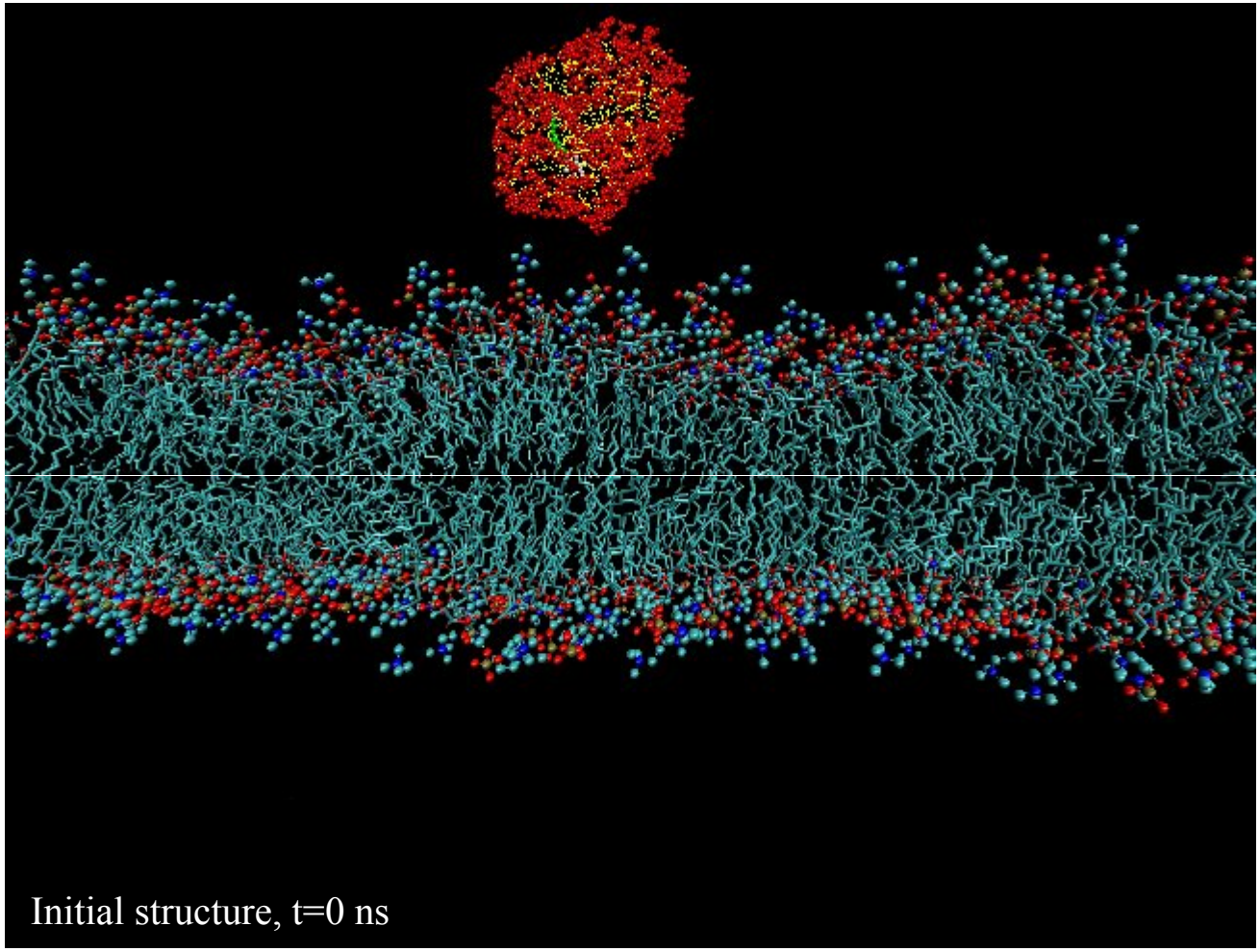
Three snapshots depicting the spontaneous self-assembly process of 125 Span80 molecules into a single spherical micelle. In the initial structure the surfactant molecules are uniformly distributed in water. For clarity the water molecules are not shown. Red spheres are used to represent the hydrophilic head groups and yellow lines represent the hydrophobic tail groups.

Encapsulation of vitamin E into Span 80 micelles



Four snapshots delineating with atomistic details the passive absorption and entrapping of an alpha-tocopherol (vitamin E) molecule into a pre-assembled Span80 spherical micelle consisting of 125 surfactant molecules. Initially the vitamin E molecule was placed at a distance of about 0.8 nm away from the surface of the micelle. In the representation of the vitamin E the silver spheres are used to represent the hydrophilic head groups and green spheres represent the hydrophobic tail groups

Transfer of a vitamin E molecule, (encapsulated in a Span 80 micelle) across a DMPC lipid bilayer



Snapshot delineating with atomistic details the setup of the simulation system used in the investigation of the interaction and transfer across a phospholipid bilayer membrane (DMPC bilayer) of vitamin E. The vitamin E was previously encapsulated into a Span80 spherical micelle. For clarity, water molecules are not shown

Conclusions (experimental)

- SYNTHESIS
  - 0.6 w/v% chitosan was found optimum to yield stable, spherical, and positively charged reproducible Chi/PLGA particle
- STABILITY
  - PLGA particles were stable under fed gastric, and all intestinal conditions for 24 hrs (but unstable under basal gastric conditions)
  - Chi/PLGA particles were stable under all gastric for 24 hrs, and fed intestinal conditions for 6 hrs (but unstable under basal intestinal conditions)
- RELEASE
  - Chi/PLGA particles released most of the alpha-tocopherol in the intestine and only half of the amount entrapped was released in 5 days
- UPTAKE
  - PLGA and Chi/PLGA particles were effectively taken-up by rabbit intestinal tissue
  - Chi/PLGA clumping may be responsible for lower uptake of particles, results which must be confirmed quantitatively

Conclusions (MD simulations)

- Using all-atom large-scale MD simulations we have shown that Span 80 aggregates spontaneously in water into spherical micelles.
- The MD simulations indicate that the aggregation process occurs in three stages: first the surfactant molecules aggregate in small clusters followed by a cluster ripening stage and ending with a slow stage involving diffusion and coalescence of small micelles.
- Our MD studies indicate that vitamin E incorporates spontaneously into a pre-assembled Span80 spherical micelle. The vitamin E encapsulation into a micelle occurs via a two-step process. First the hydrophobic tail of the molecule is incorporated into the surface of the micelle followed by reorientation and penetration of the tail into the tails region of the micelle.

Future work

- Fluorescent PLGA-TRITC will be synthesized
- Fluorescent PLGA and Chi/PLGA nanoparticles of different characteristics (size, surface potential) with entrapped alpha-tocopherol will be synthesized
- *In-vivo* mucoadhesion and uptake studies will be performed
- MD simulations of transport of vitamin E across a lipid bilayer will be completed