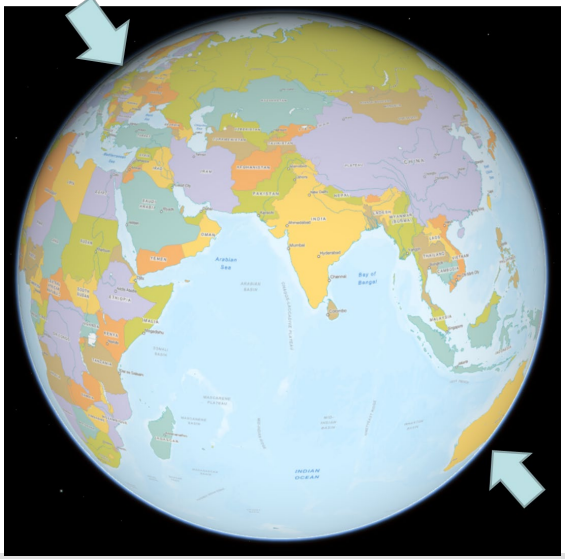
MONASH University
Institute of Pharmaceutical Sciences

UNIVERSITY OF COPENHAGEN



SAXS on *(food related)* soft matter during digestion

Ben J. Boyd and many others

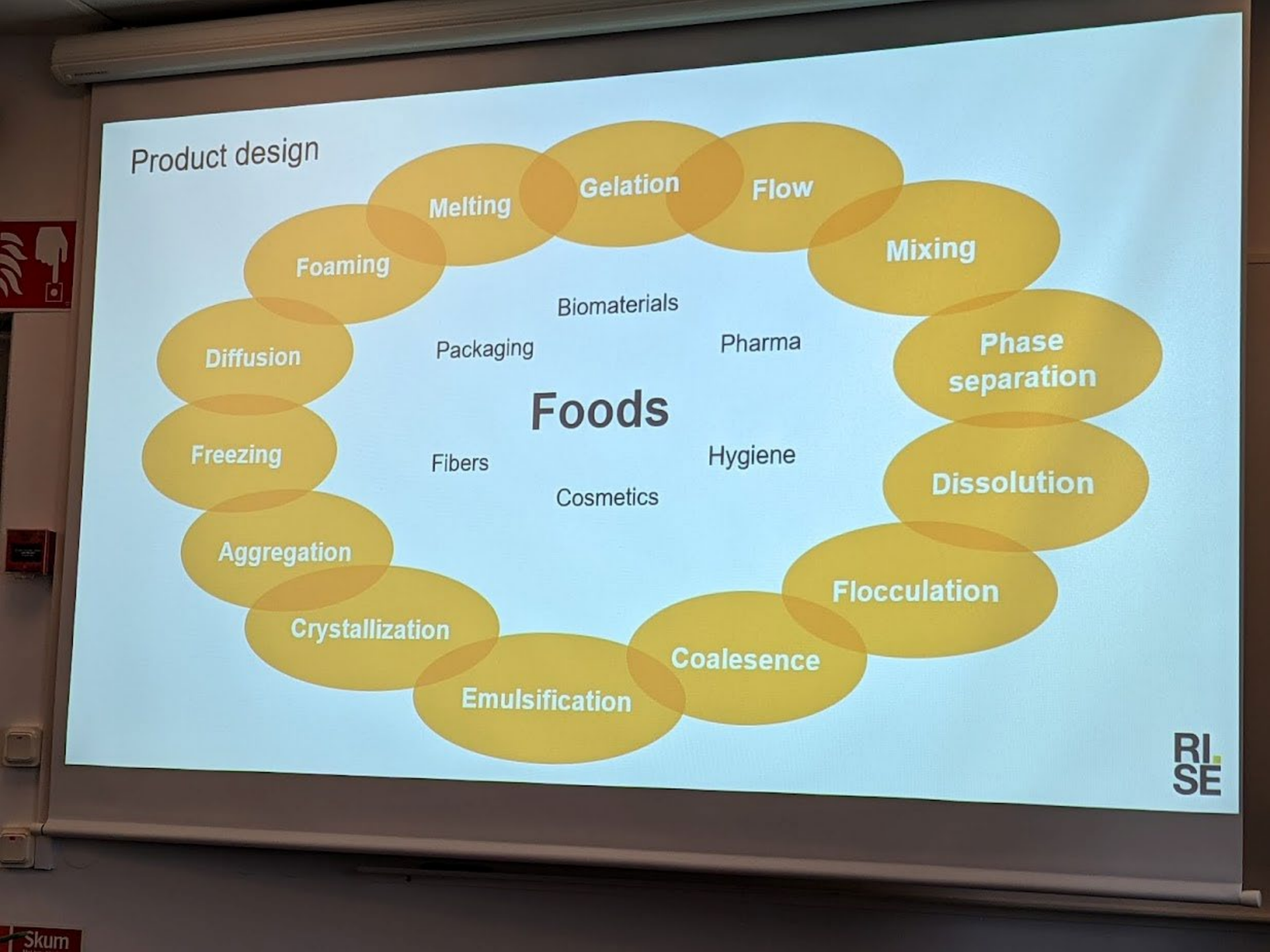
Department of Pharmacy – University of Copenhagen

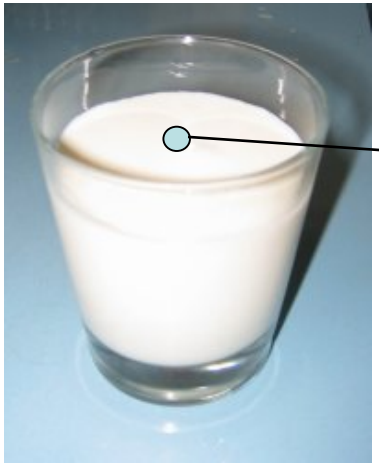
Monash Institute of Pharmaceutical Sciences

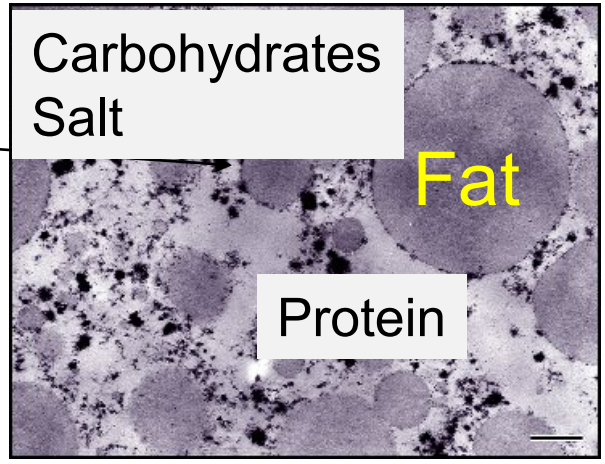
ben.boyd@sund.ku.dk

LINXS Masterclass November 2021

@group_boyd or nonlamellar.com


Product design
Melting
Gelation
Flow
Foaming
Mixing
Biomaterials
Diffusion
Packaging
Pharma
Phase separation
Foods
Freezing
Fibers
Hygiene
Dissolution
Cosmetics
Aggregation
Flocculation
Crystallization
Coalesence
Emulsification
RI. SE

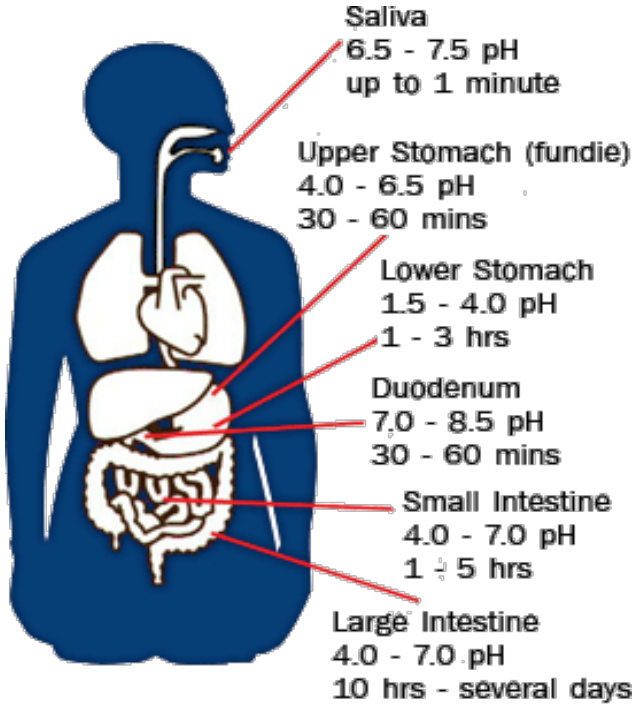




Temperature
pH
Processing history

Consider milk as a prototypical food!

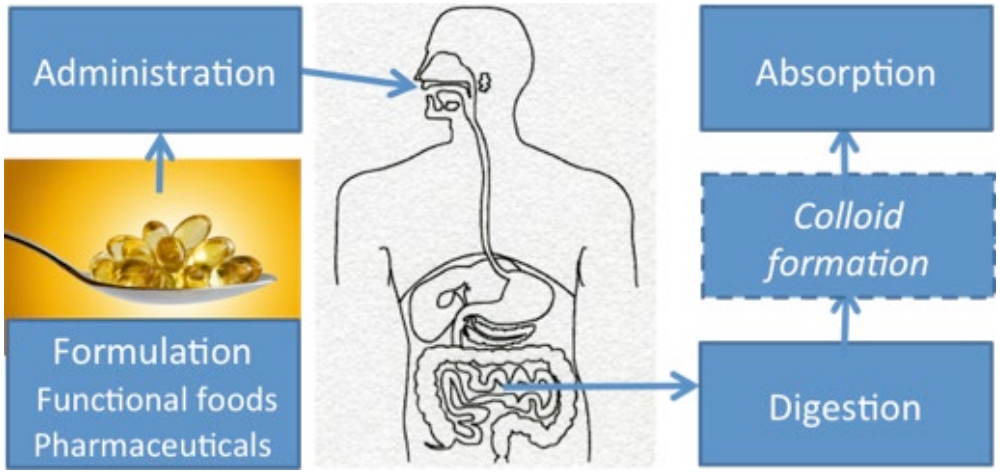
Digestion



This diagram illustrates the average time food spends in each part of the digestive system along with the average pH.

Enzyme	*Substrate*	*Products*
Lipases	Triglycerides	monoglycerides and fatty acids
Phospholipases	Phospholipids	lysophospholipids + fatty acids
Amylase	Carbohydrates (starch, alginates)	starch fragments/monosaccharides
Lactase	Lactose	galactose and glucose
Proteases	Proteins e.g. casein	peptides and amino acids

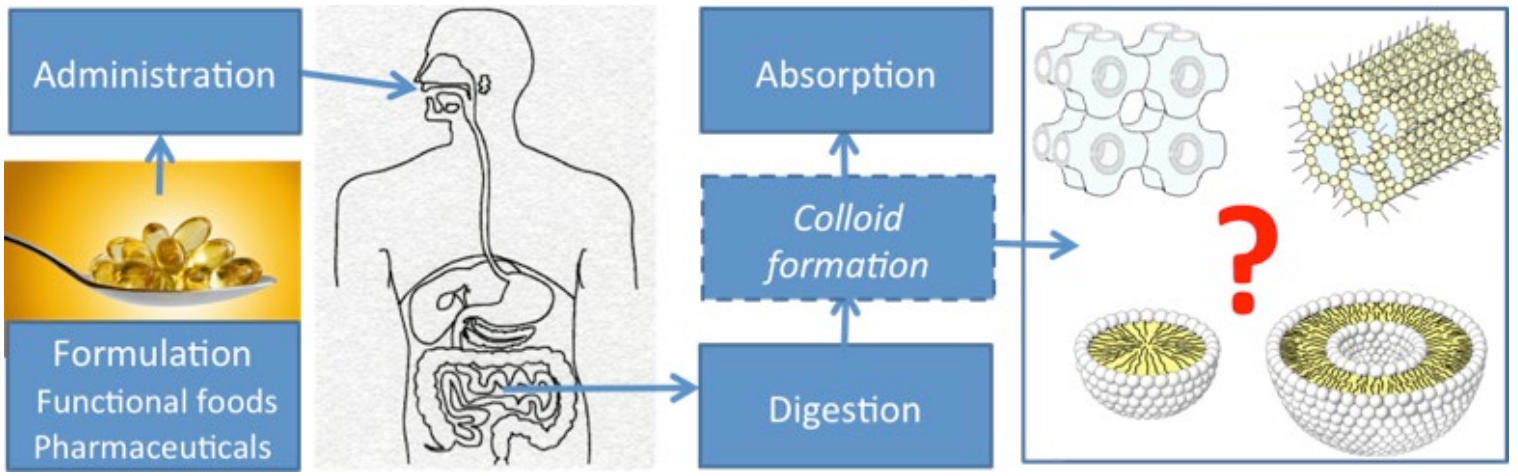
Digestion may lead to changes in structure

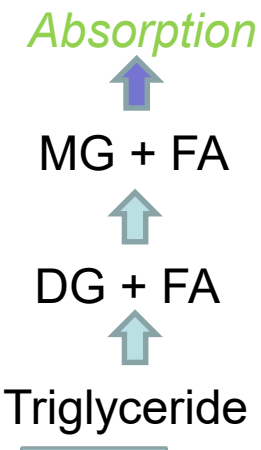


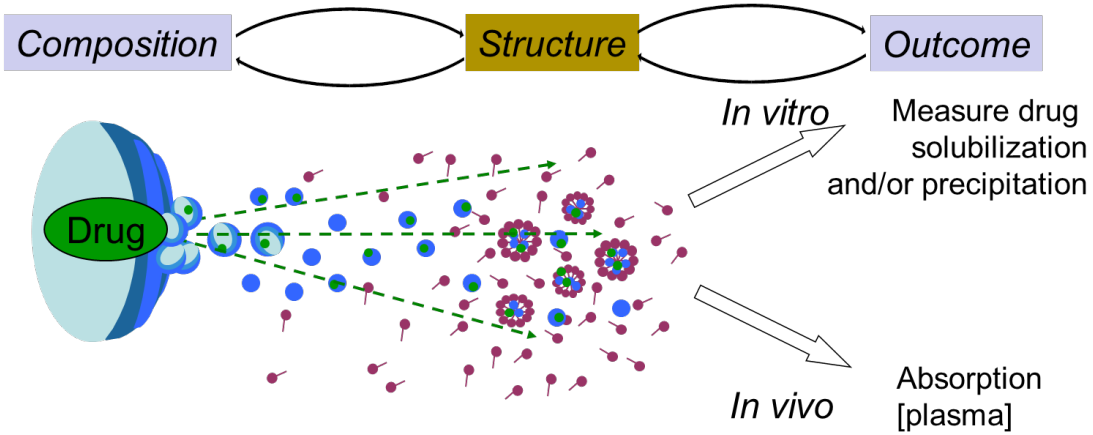
Changes in structure due to digestion may lead to:

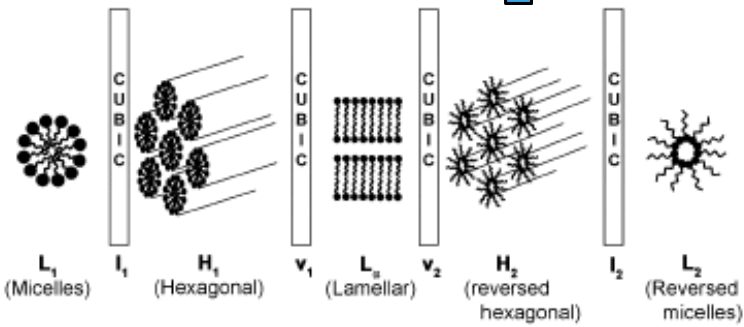
1. Changes in rate of absorption of nutrients (pharmacokinetics)
2. Changes in extent of absorption of nutrients (bioavailability)
3. Changes in reaction of the body to food (e.g. immunogenicity)
4. Separation of components (demixing, coacervation)
5. Degradation/enhanced stability (unintended release)

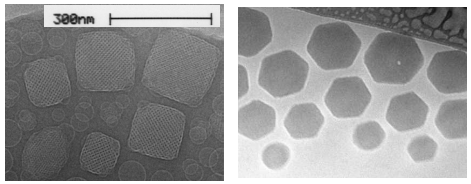
Structure in the context of lipids – hierarchical structure











Cubosomes Hexosomes

Kaasgaard and Drummond, Phys. Chem. Chem. Phys., 2006, 8, 4957–497

Lipase added to MO cubic phase

100 mM phosphate,
starting pH = 8
Fixed sample in capillary

Langmuir **2002,** *18,* 8972−8981

Effect of Lipase on Different Lipid Liquid Crystalline Phases Formed by Oleic Acid Based Acylglycerols in Aqueous Systems

Johanna Borné, Tommy Nylander,* and Ali Khan

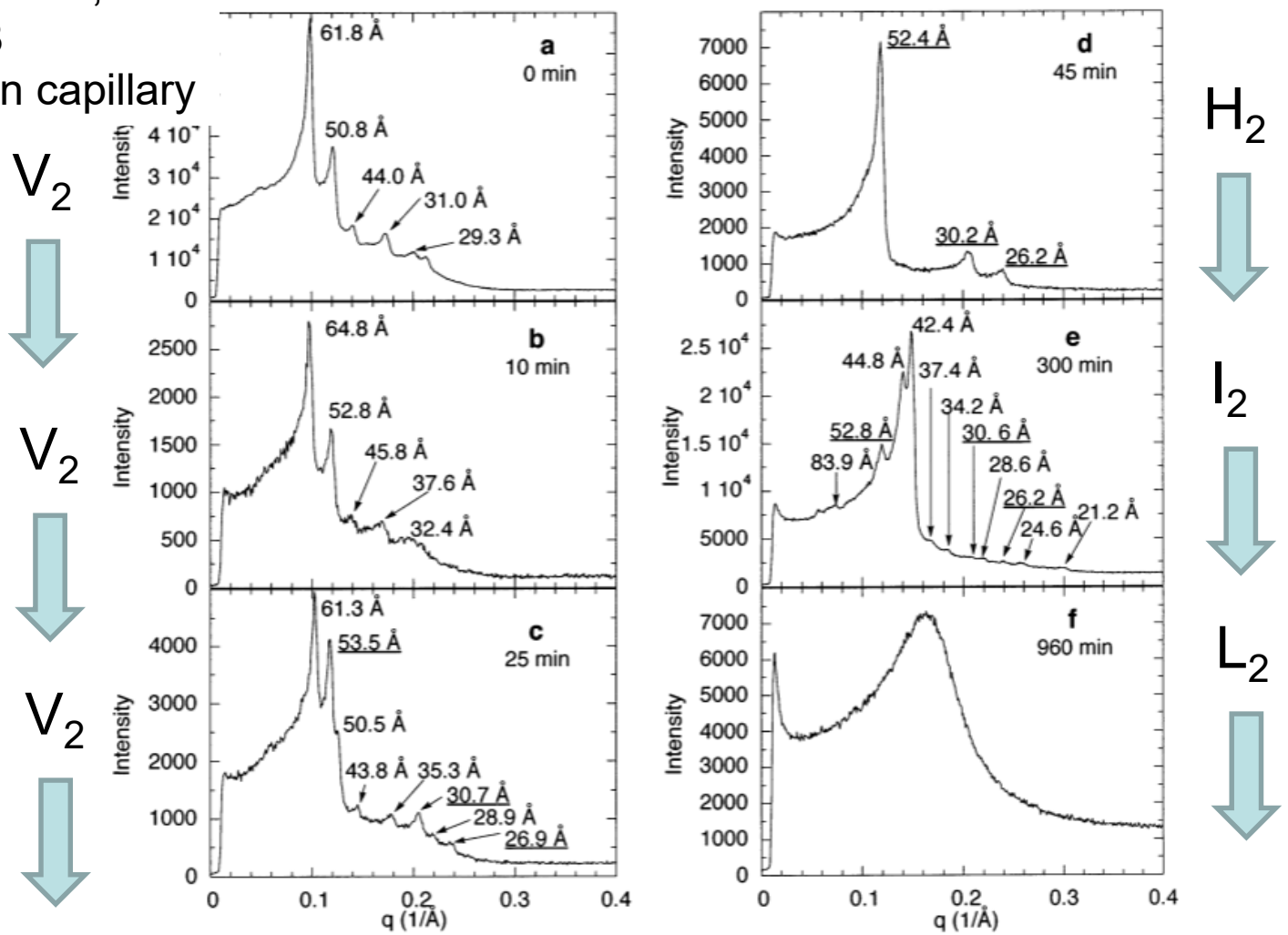
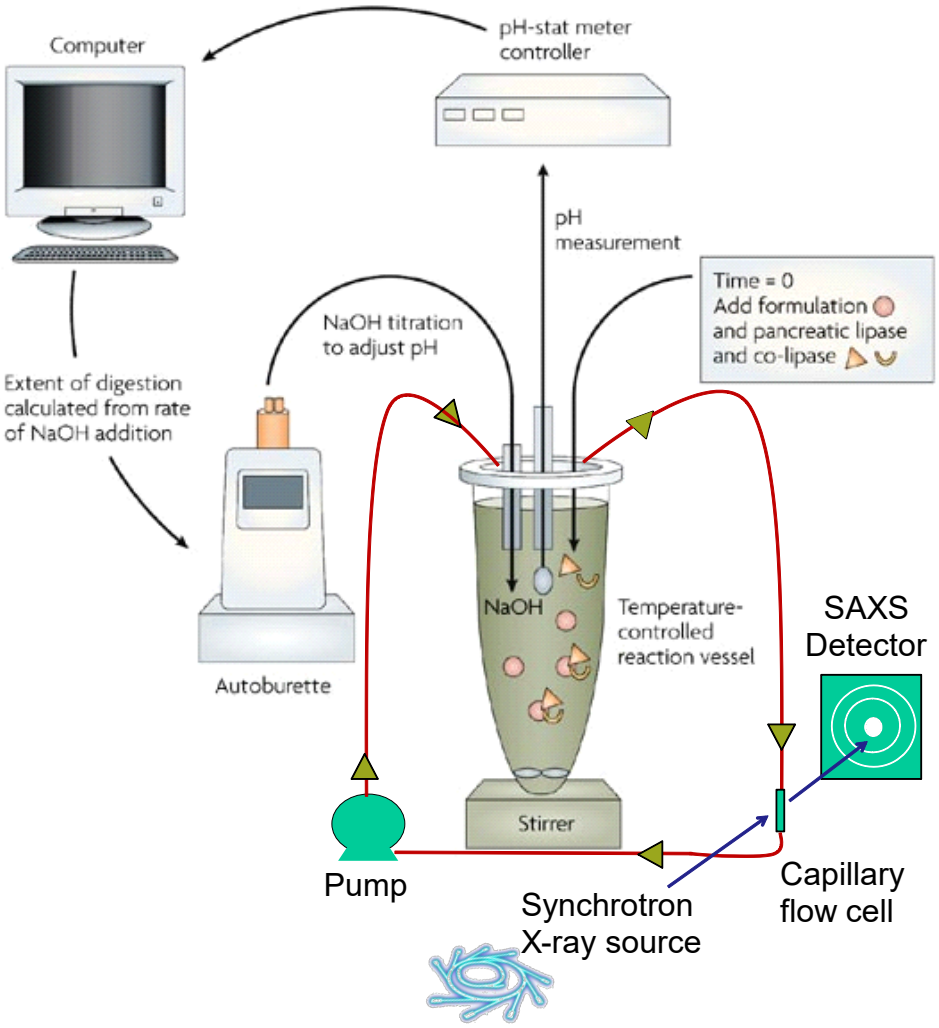
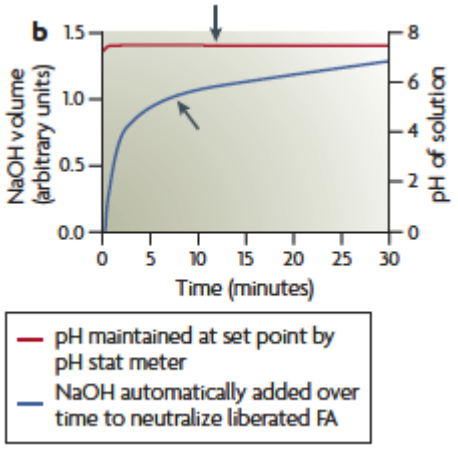
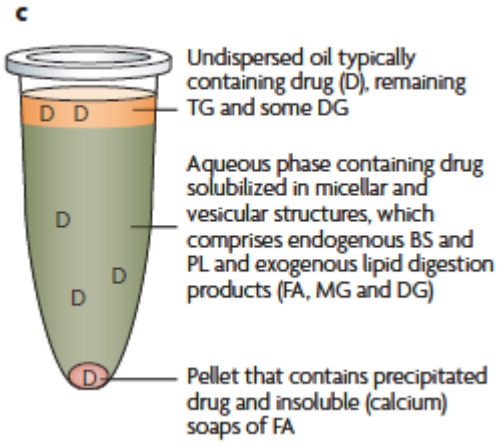


Figure 2. SAXD data recorded after adding 20 μL of (512 nM) TLL solution to 30 mg of C_D phase (63 wt % MO, 37 wt % ^2H_2O) at 25 °C, giving a final TLL concentration of 205 nM. The intensity is plotted versus the wave vector, $q = 2^*\pi/d$, where d is the spacing between the lattice planes. The sequence of phase transitions observed is (a) C_D (0 min), (b) C_D (10 min), (c) $C_D + H_{II}$ (25 min), (d) H_{II} (45 min), (e) $C_{mic} + H_{II}$ (300 min), and (f) L_2 (960 min). The d values for the reflections corresponding to the H_{II} phase have been underlined in the figure.

Lipid digestion model with time resolved scattering



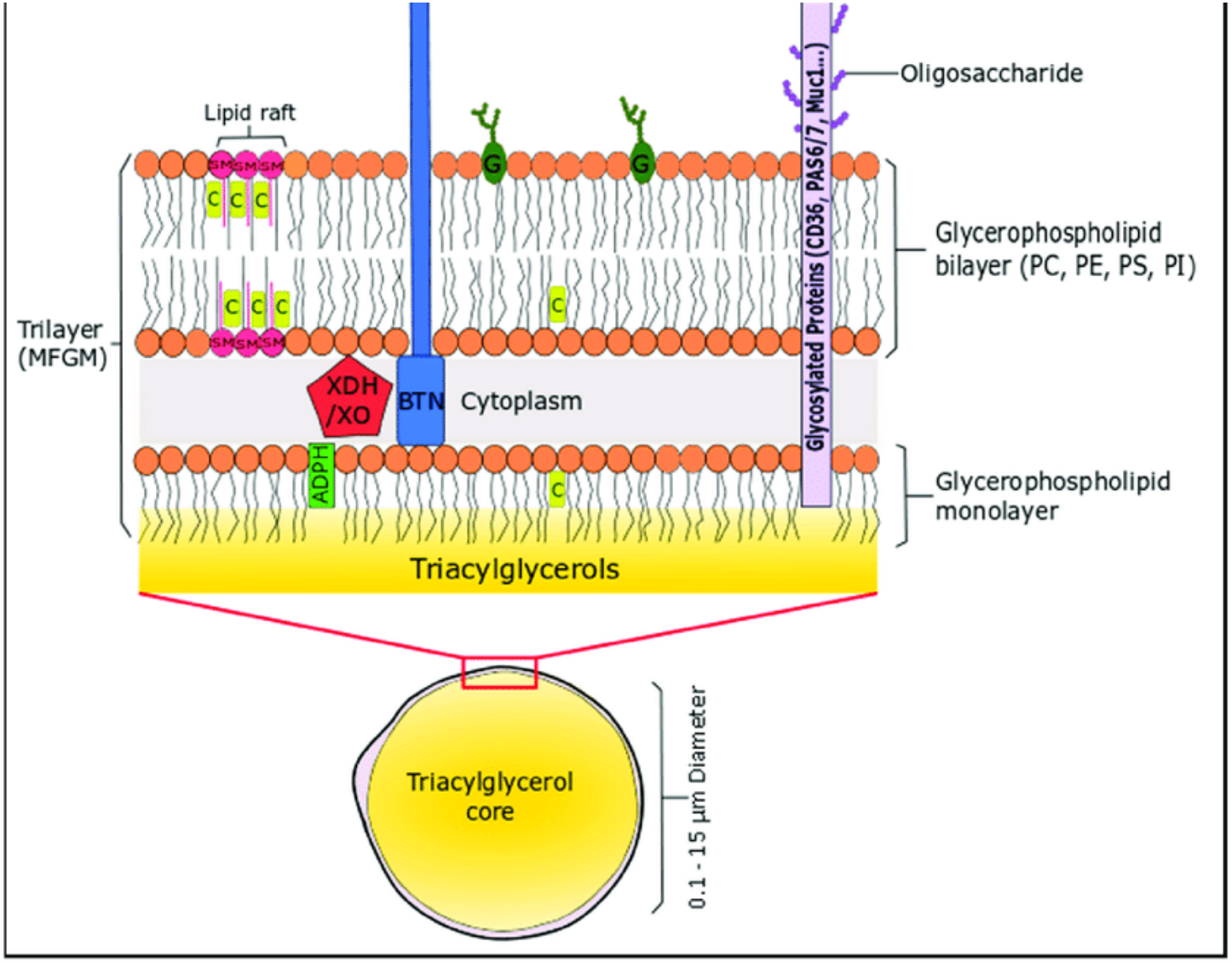




Time-resolved structural information during digestion

Figure from Porter, C.J.H., N.L. Trevaskis, and W.N. Charman, *Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs.* Nature Reviews Drug Discovery, 2007. **6**(3): p. 231-248.

‘Structure’ of milk fat droplets



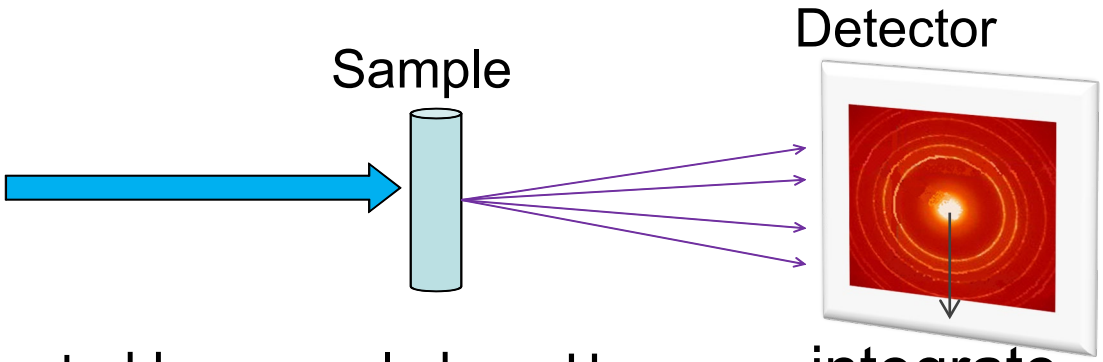
- Anticipate triglycerides will dominate structural changes upon digestion?

Image Lordan et al. DOI - 10.3390/molecules22111964

Characterization – small angle X-ray scattering (SAXS)

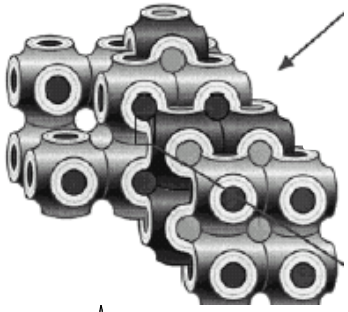
X-ray source

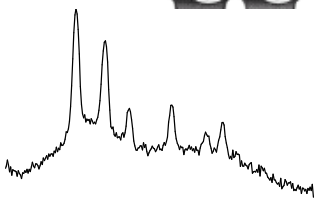
Lab/synchrotron



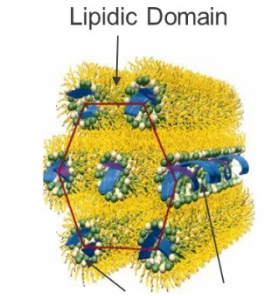
integrate

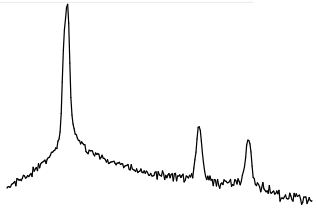
Cubic phase - V_2



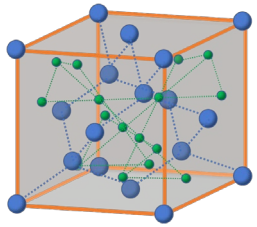


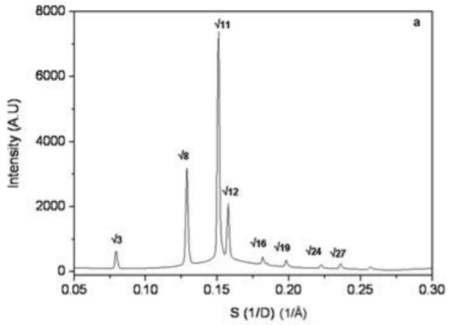
Inverted hexagonal phase H_2





Inverted micellar cubic phase I_2

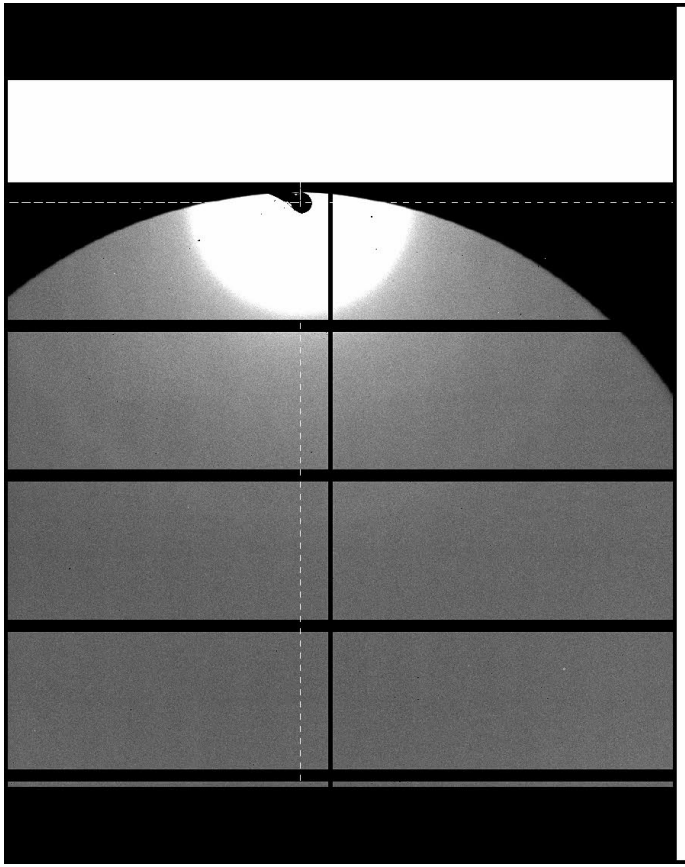


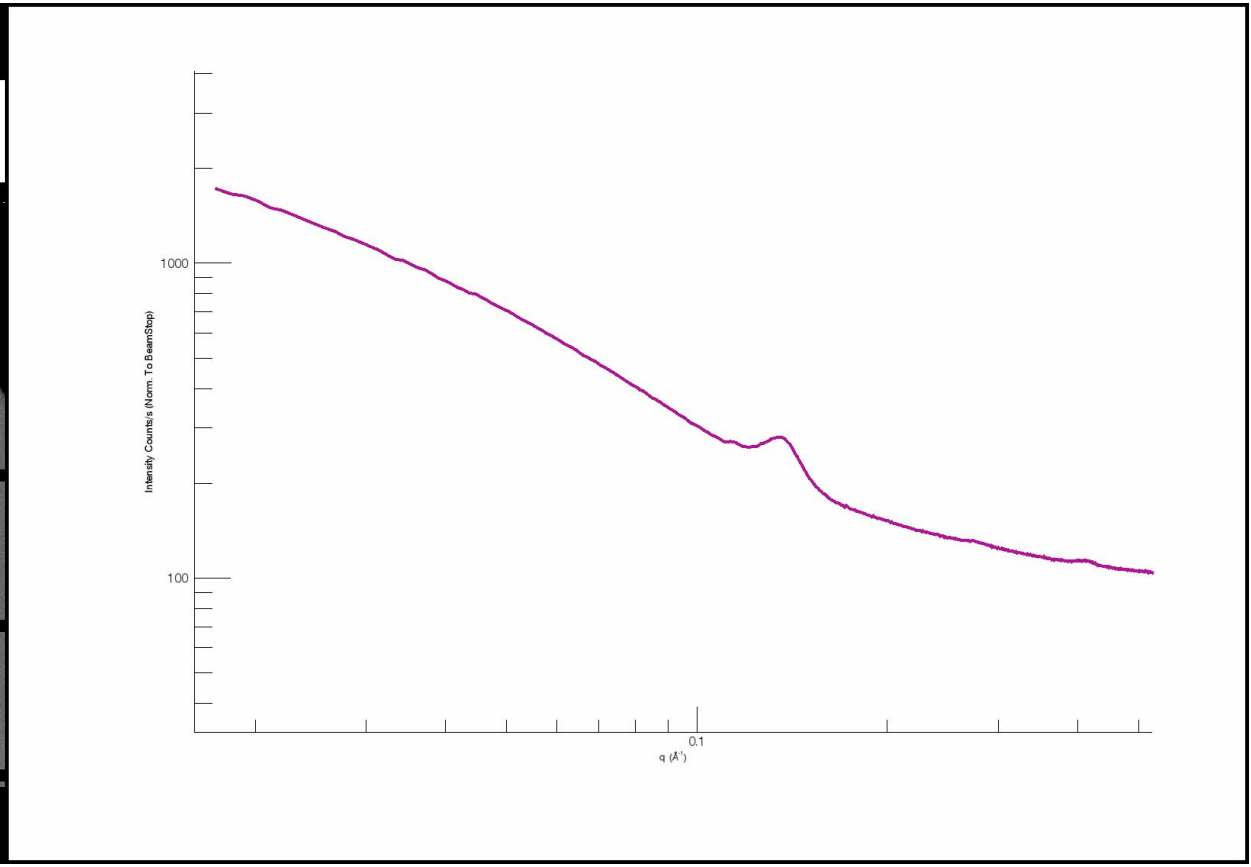


Scattering is a ‘fingerprint’ for internal phase structure

What do we ‘see’?

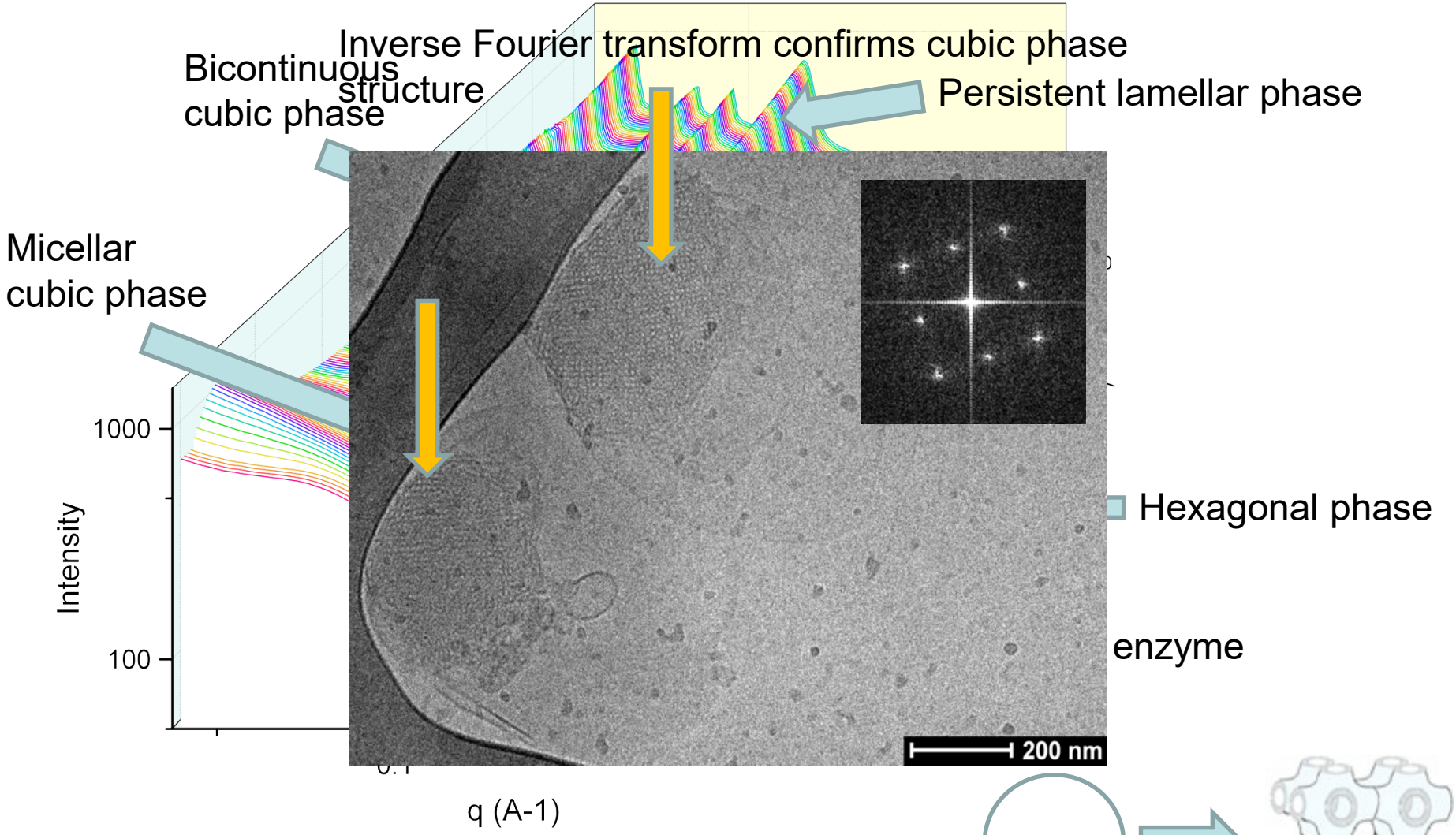
Milk ! – The Movie





- Complex dynamic transformations in structure

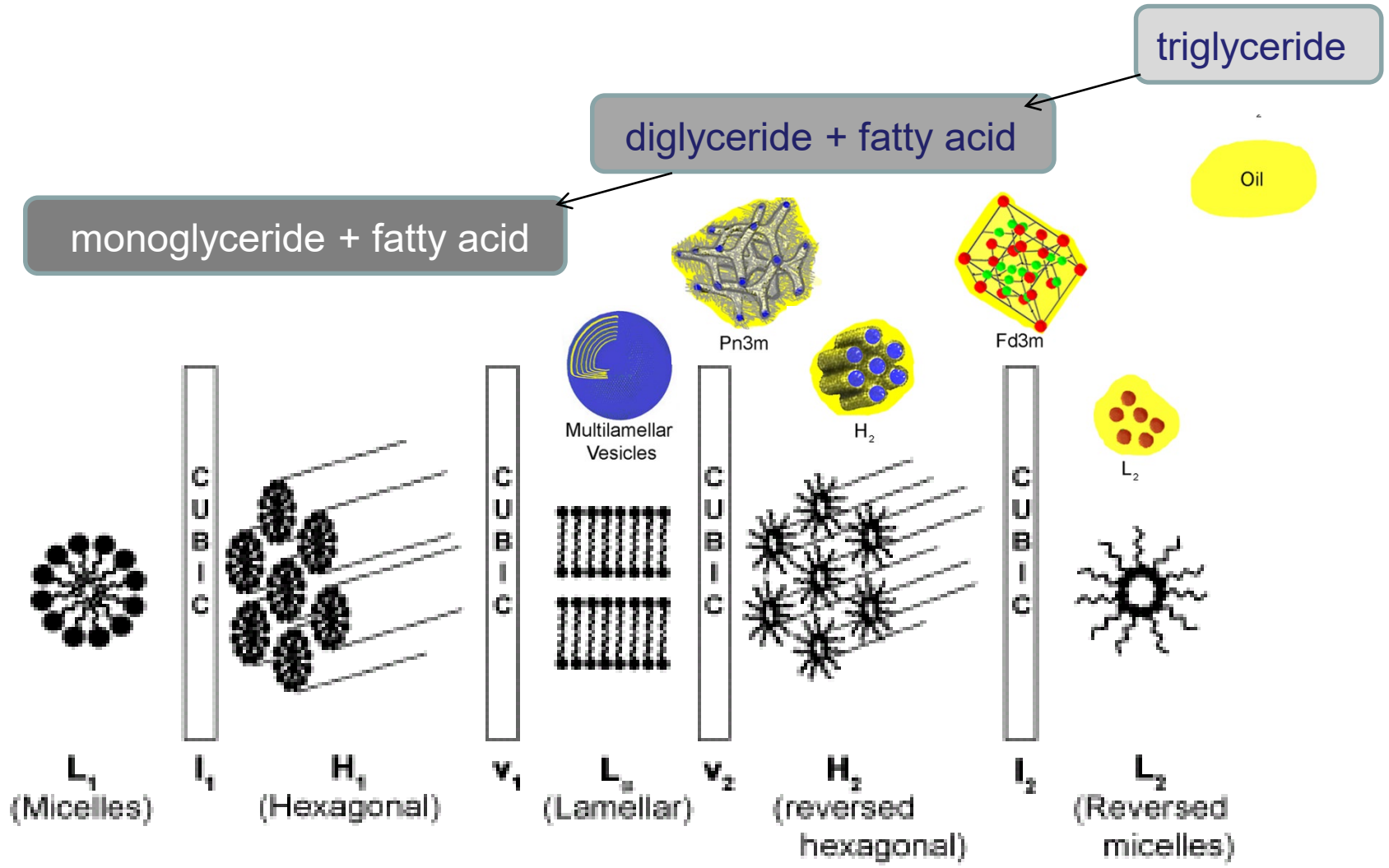
What do we 'see' when we digest milk?



- Structures formed from unstructured emulsion
- Highly dynamic system during digestion
- Droplets transform from liquid to 'cubosomes'

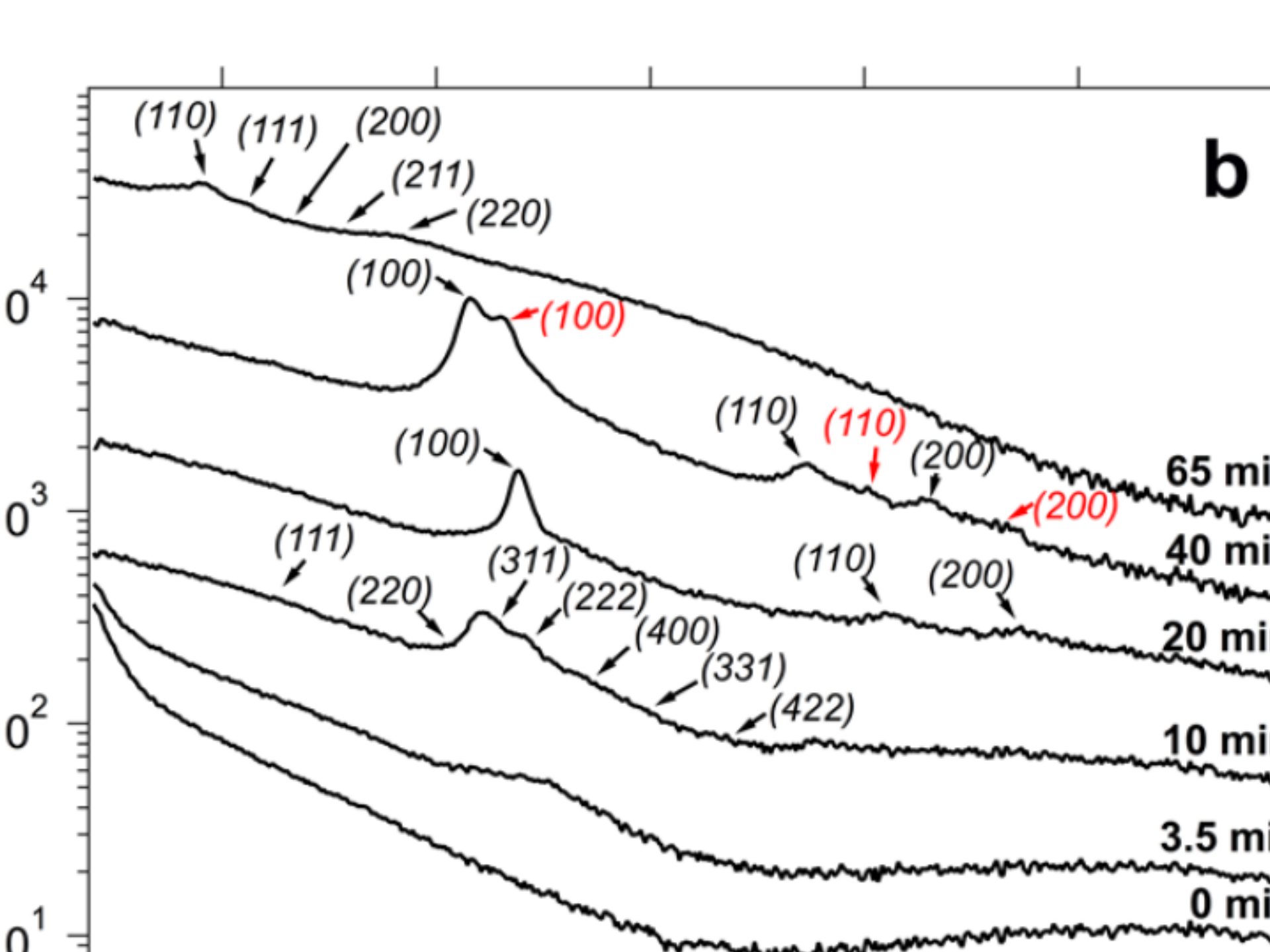
Salentinig et al. ACS Nano 2013

Digestion induces systematic change in lipid packing



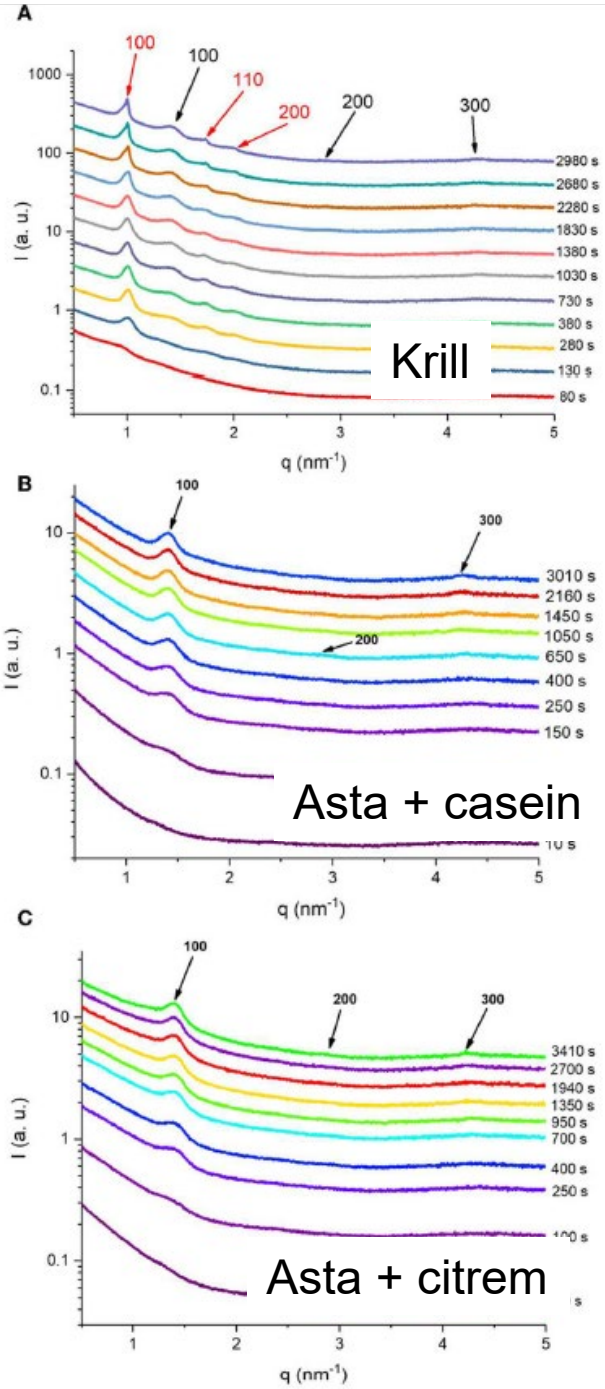
"Makes sense" from a physical chemistry/packing parameter perspective

Kaasgaard and Drummond, Phys. Chem. Chem. Phys., 2006, 8, 4957–497


b
(110)
(111)
(200)
(211)
(220)
(100)
(100)
(110)
(110)
(200)
(200)
65 mi
(100)
(111)
(220)
(311)
(222)
(400)
(331)
(422)
(110)
(200)
40 mi
20 mi
10 mi
3.5 mi
0 mi
0^4
0^3
0^2
0^1

Krill oil and astaxanthin (high ω-3 TG) oil

- Krill oil (crude mixture) contains phospholipids, self-stabilising on dispersion
- Astaxanthin oil is purified TG fraction, emulsified in casein or citrem
- Same model as before, SAXS at PSI
- Simplicity removed the structure formation



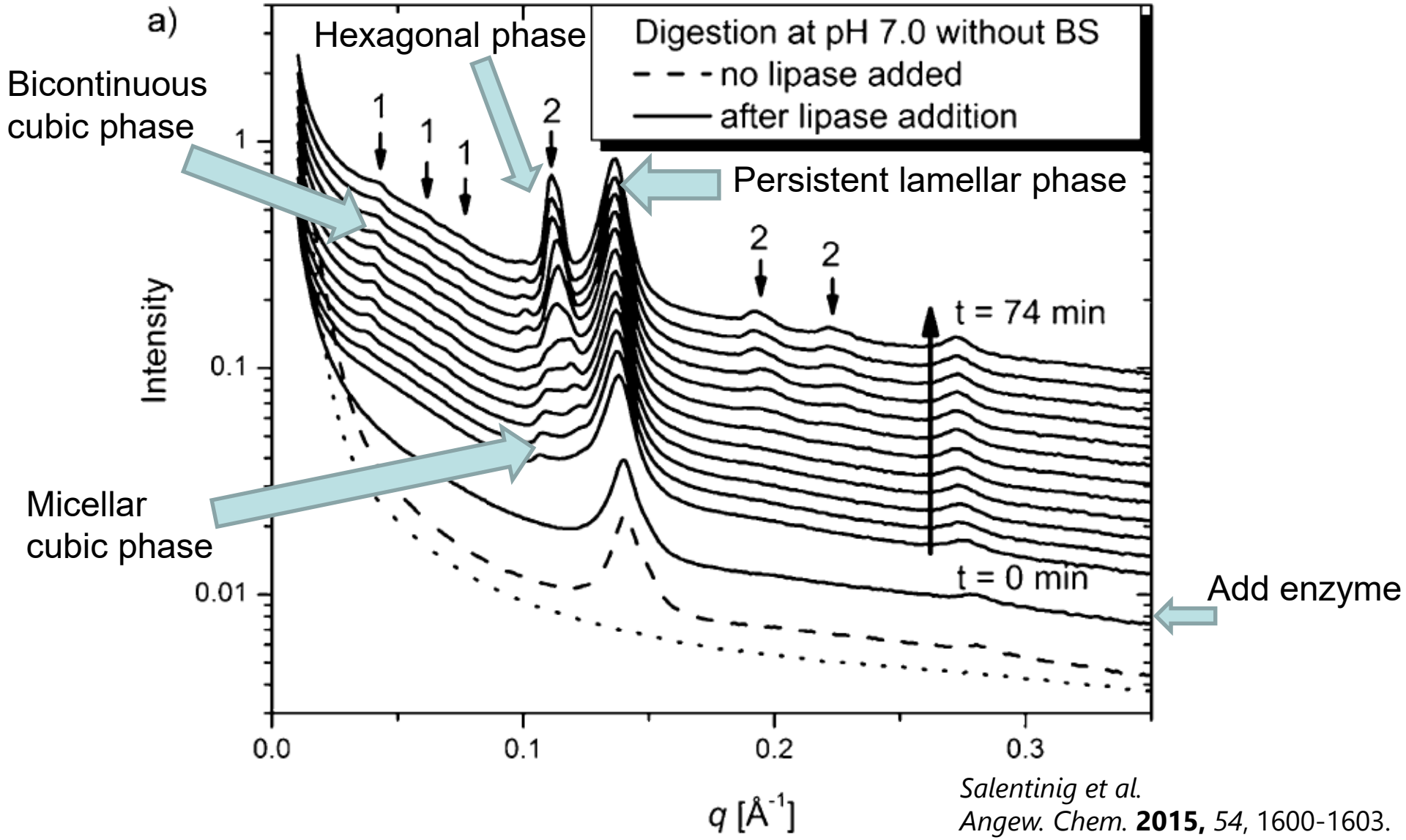
frontiers in Bioengineering and Biotechnology

ORIGINAL RESEARCH
published: 05 December 2019
doi: 10.3389/fbioe.2019.00384

Internal Lamellar and Inverse Hexagonal Liquid Crystalline Phases During the Digestion of Krill and Astaxanthin Oil-in-Water Emulsions

Anan Yaghmur[1], Saleh Lotfi[1†], Sarah Atoussa Ariabod[1†], Gizem Bor[1], Mark Gontsarik[2] and Stefan Salentinig[2,3]**

What do we 'see' when we digest human breast milk?

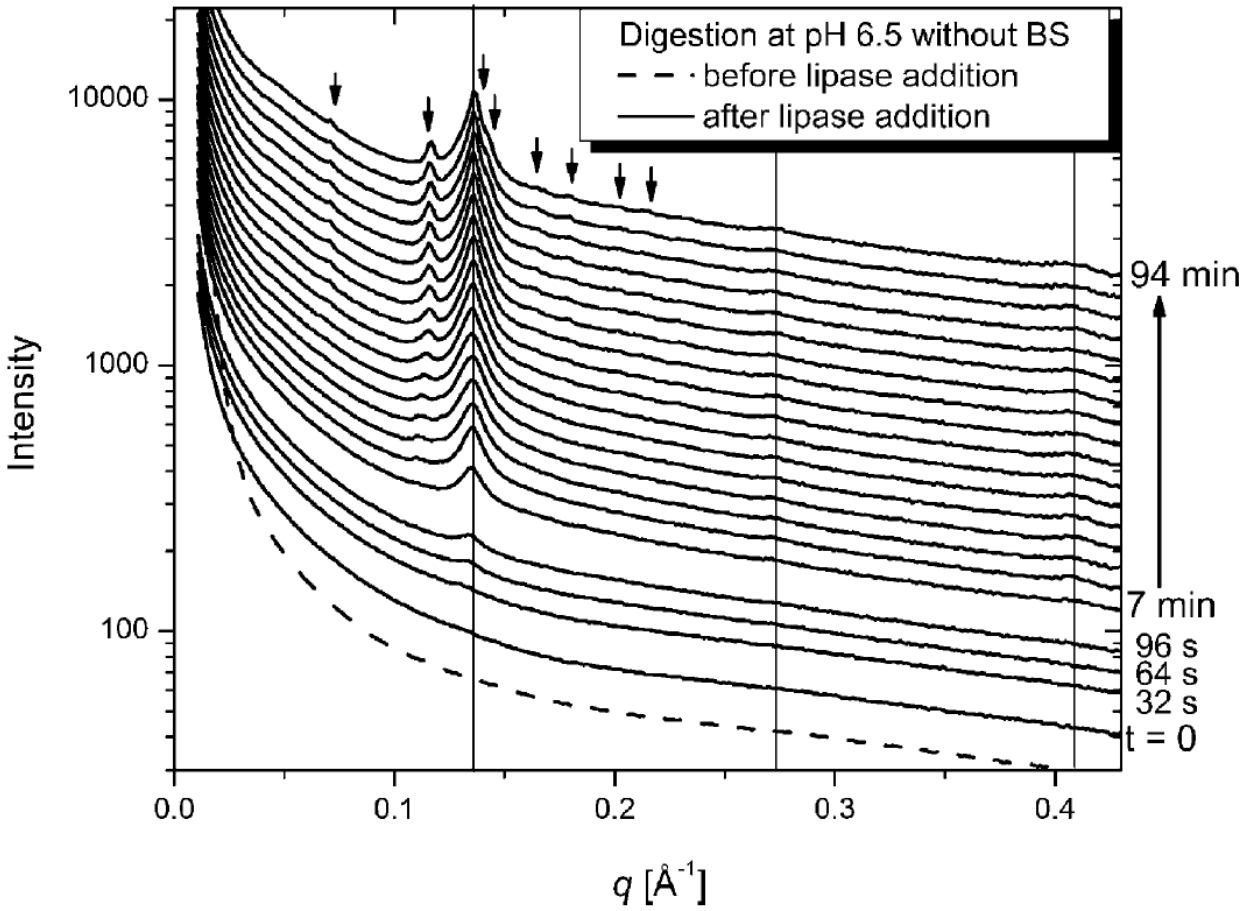


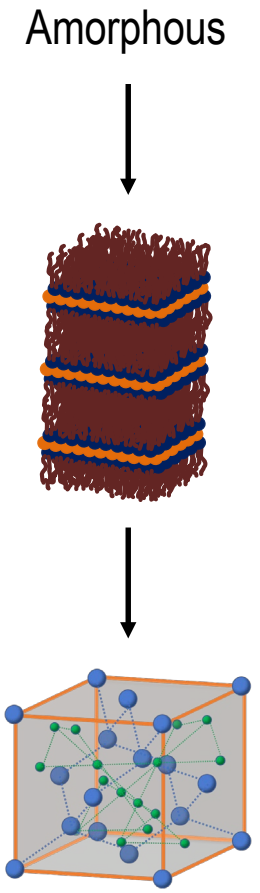
Salentinig et al.
Angew. Chem. **2015,** *54*, 1600-1603.

- Almost identical structural transitions as bovine milk
- Human breast milk 'self-digests' at 37°C due to bile salt-stimulated lipase

pH control is very important

Donor human breast milk from
Mercy Health Breastmilk Bank





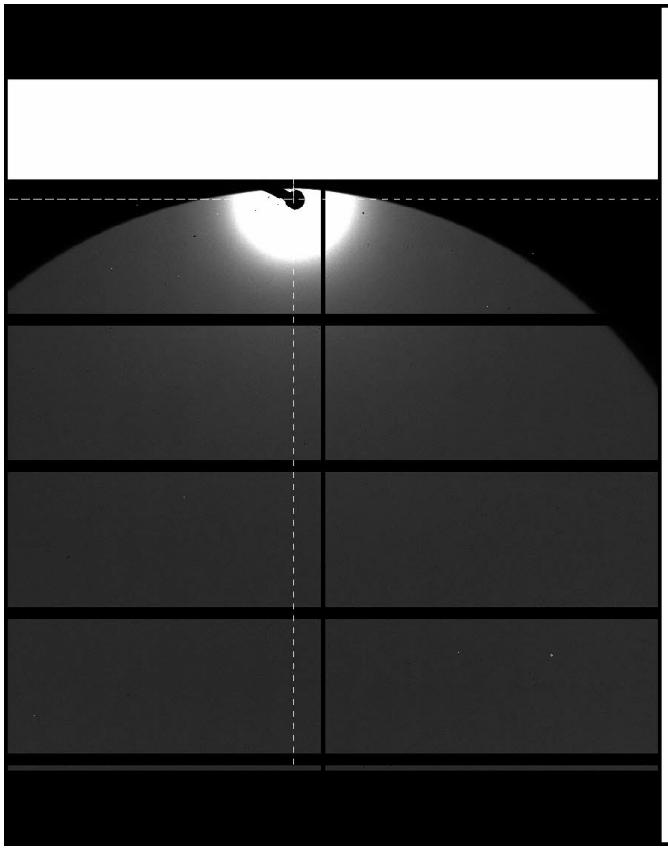
- Inverted micellar cubic phase (I_2) main non-lamellar structure

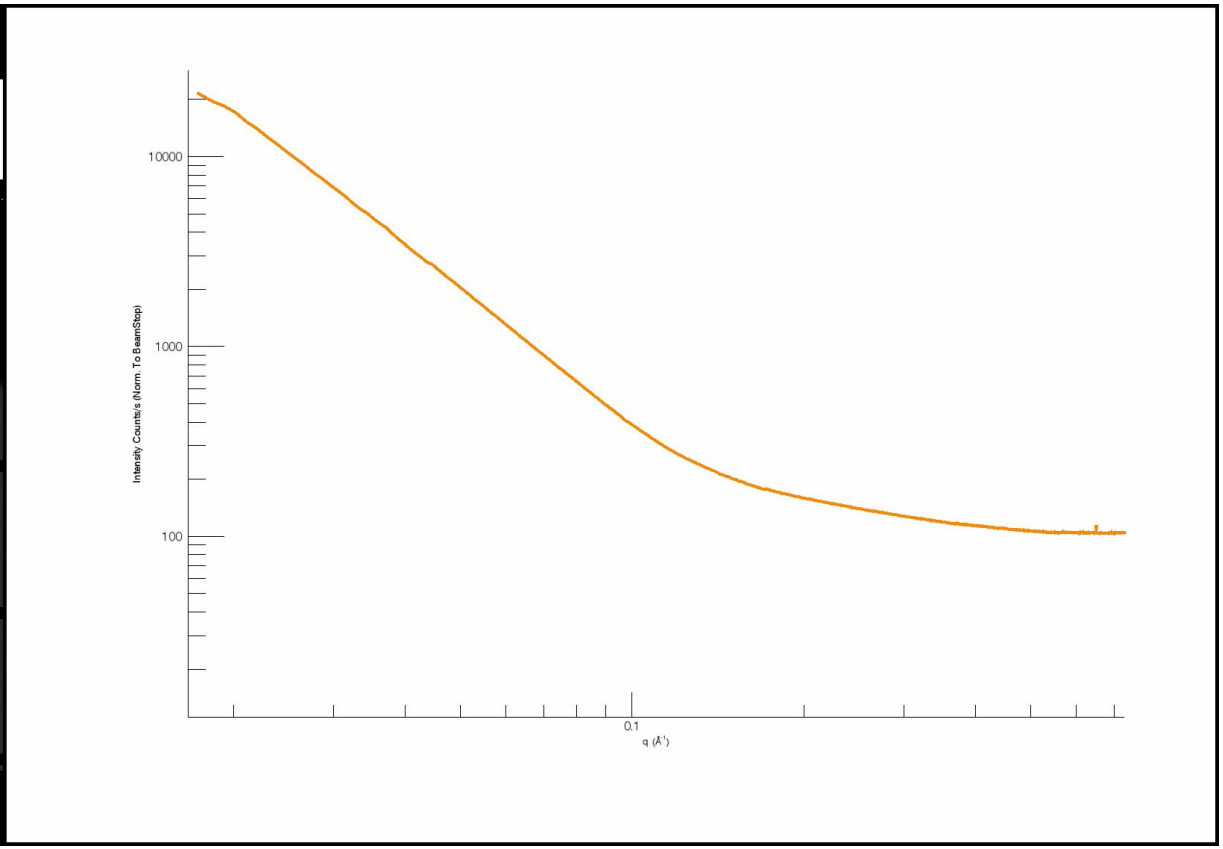
Published in Salentinig et al. Angewandte Chemie (2015), 54, 1600-1603.

Rare footage of the first wild almond to ever be milked (circa 1885)

What do we ‘see’ with other ‘so-called’ milks?

Digestion of soy milk

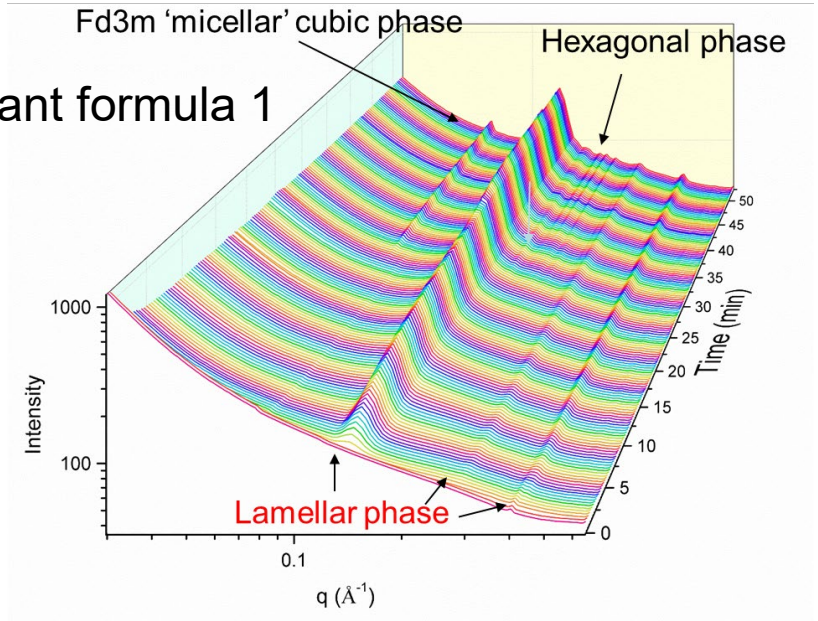




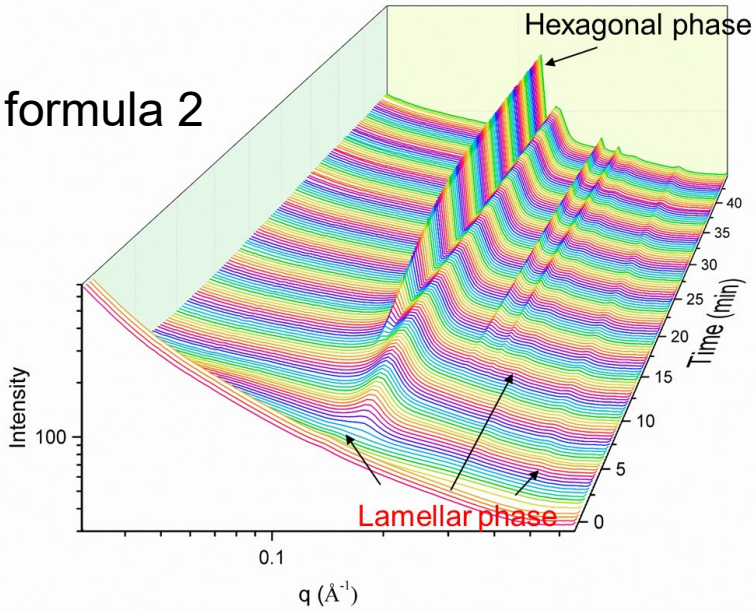
Pham et al. ACS Appl. Bio Mater. 2020, 3, 3087−3098

Infant formula can be similar, or VERY different to milk

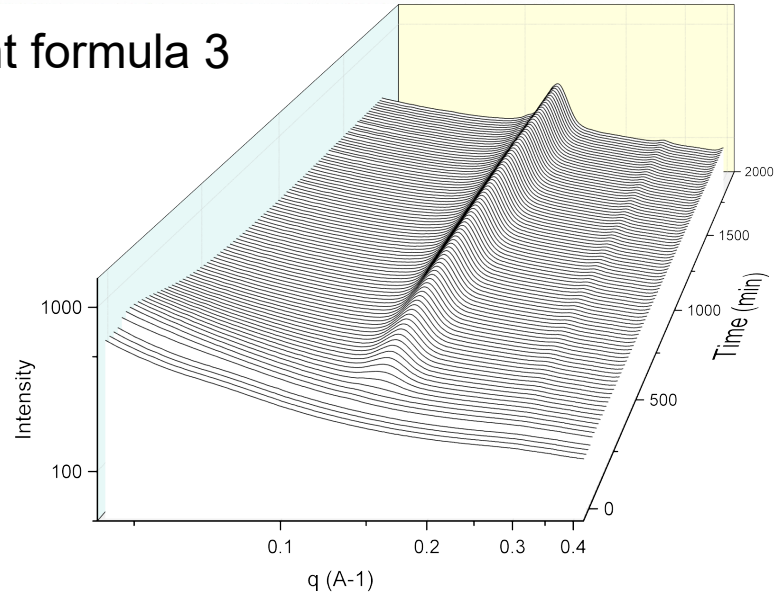
Infant formula 1



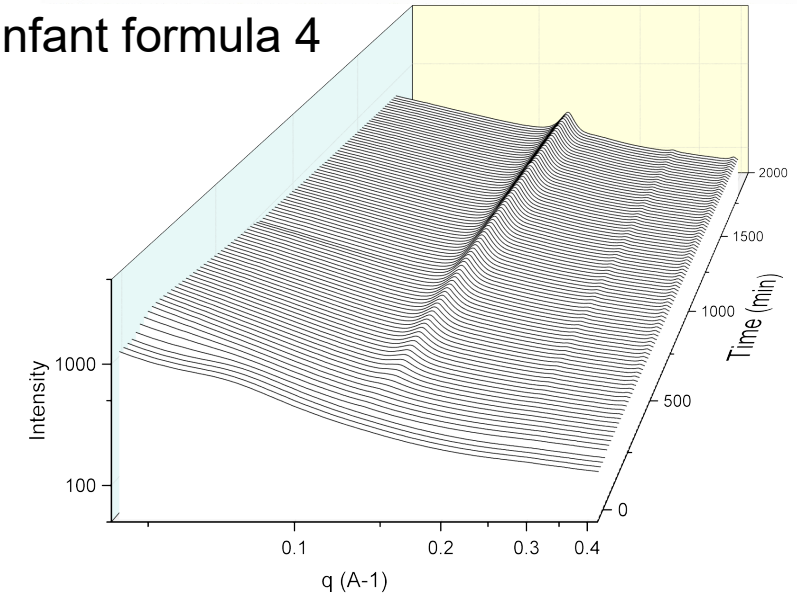
Infant formula 2



Infant formula 3



Infant formula 4

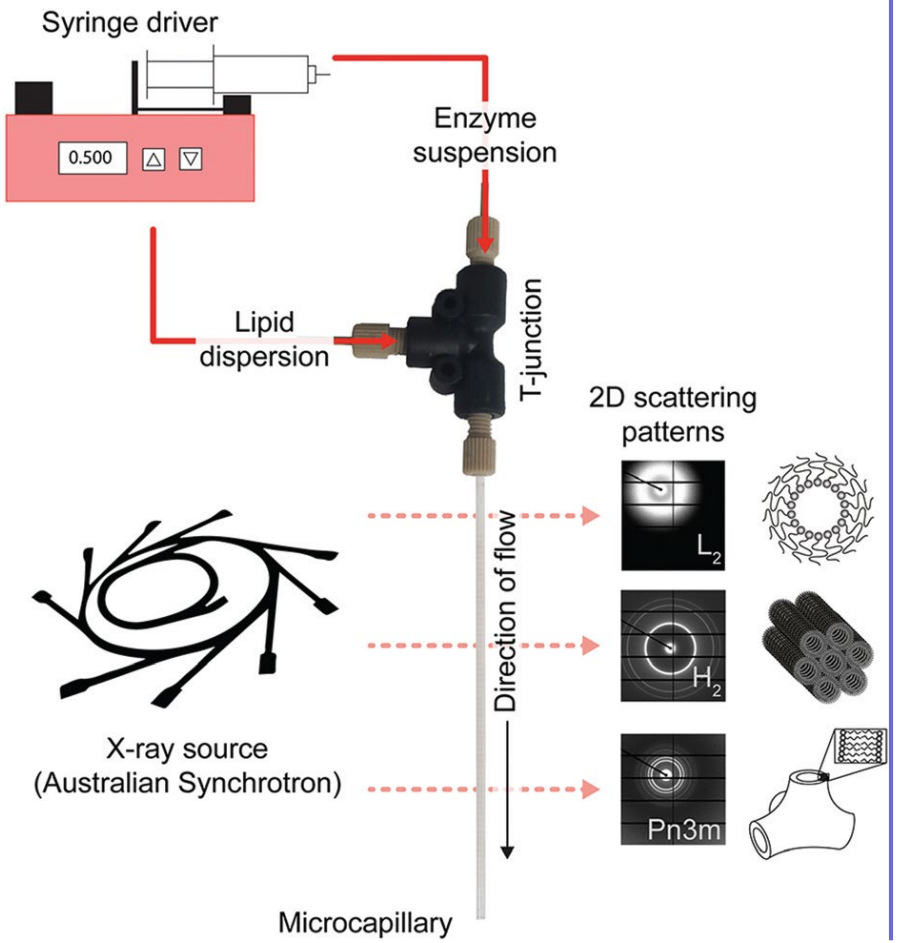


Pham et al. ACS Appl. Bio Mater. 2020, 3, 3087−3098

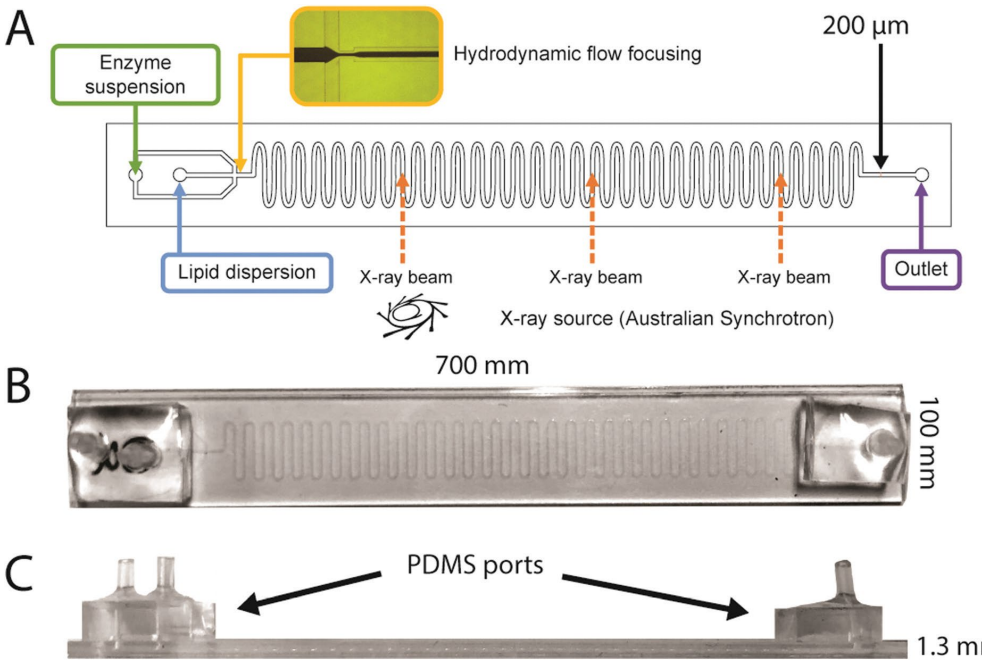
Lipid digestion and microfluidics

Linda Hong….Ben J. Boyd, Soft Matter, 2019

T-mixer



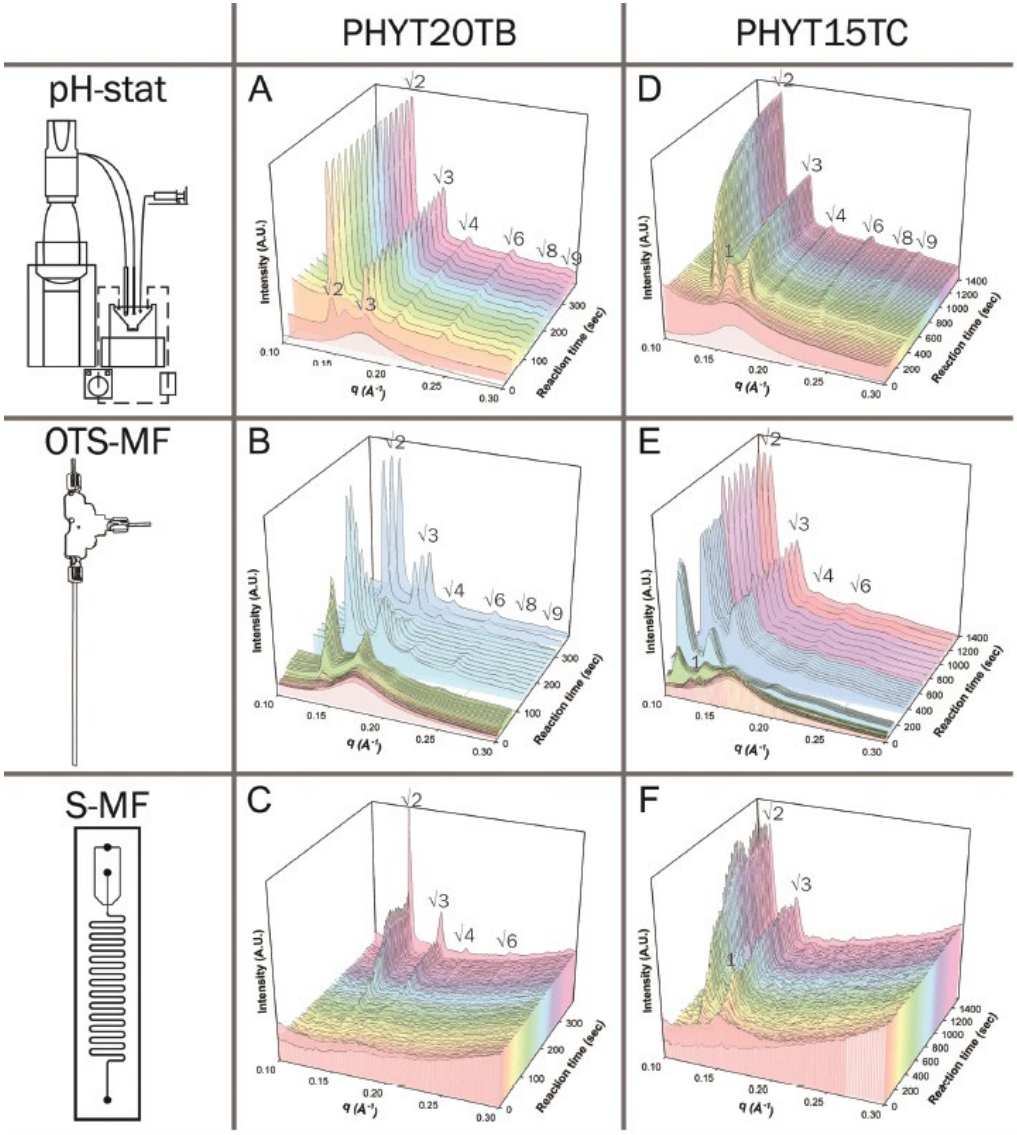
‘Serpentine’ flow focussing



Lipid digestion and microfluidics

Linda Hong….Ben J. Boyd, Soft Matter, 2019

- High throughput
- Structural changes in agreement
- Avoid radiation damage
- Spatial sampling of time
- Coupling to other techniques
- Transmission can be an issue



Structure from carbohydrates – maize starch

Carbohydrate Polymers 85 (2011) 281–293

Contents lists available at ScienceDirect

Carbohydrate Polymers

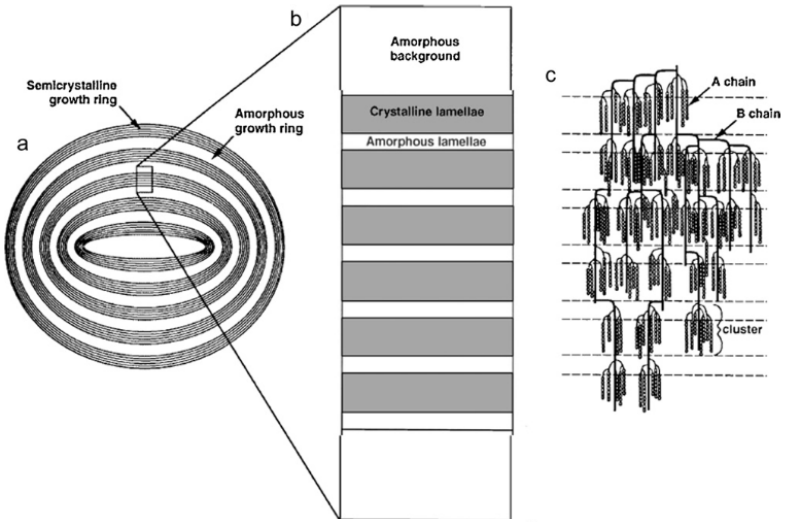
journal homepage: www.elsevier.com/locate/carbpol

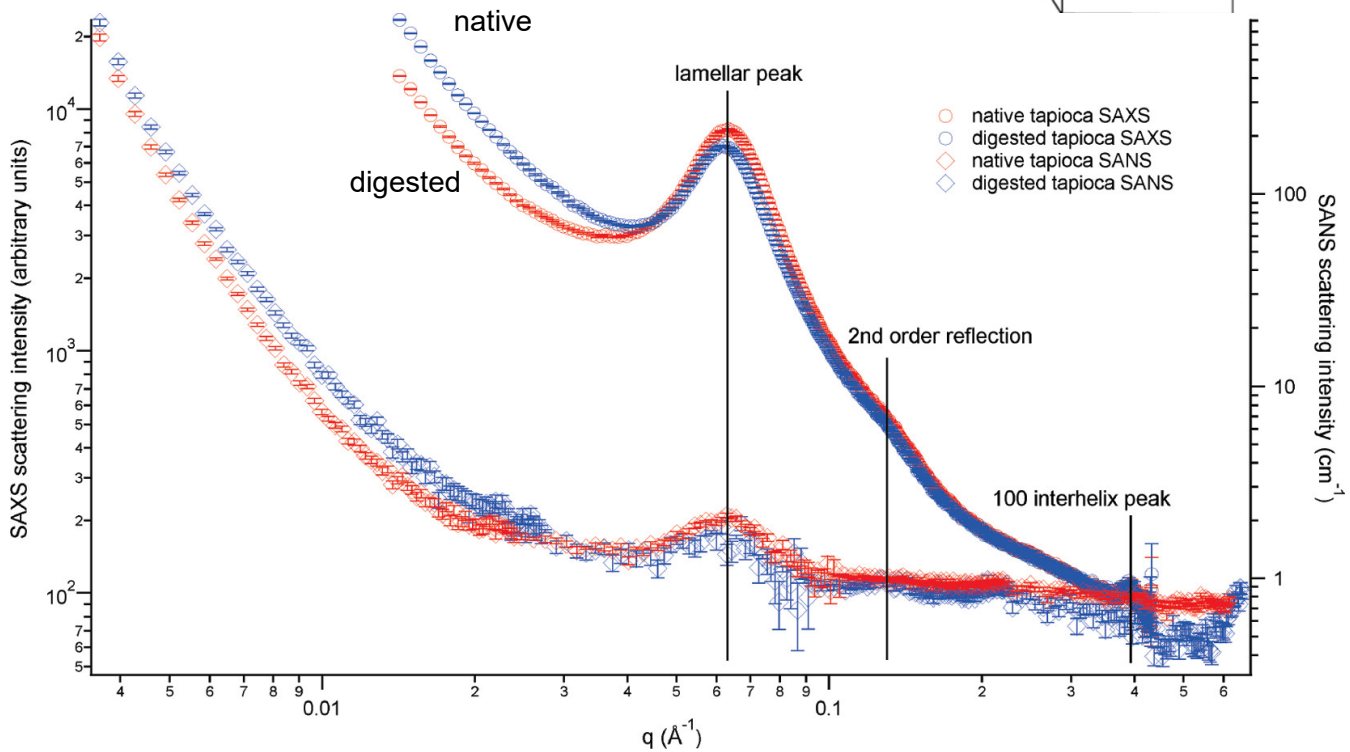
Review

Application of small-angle X-ray and neutron scattering techniques to the characterisation of starch structure: A review

Jaroslav Blazek[a,b], Elliot Paul Gilbert[a,*]

[a] Bragg Institute, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, NSW 2232, Australia
[b] Food Futures Flagship, North Ryde Riverside Corporate Park, 5 Julius Avenue, North Ryde, NSW 2113, Australia





Structure from carbohydrates – maize starch

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

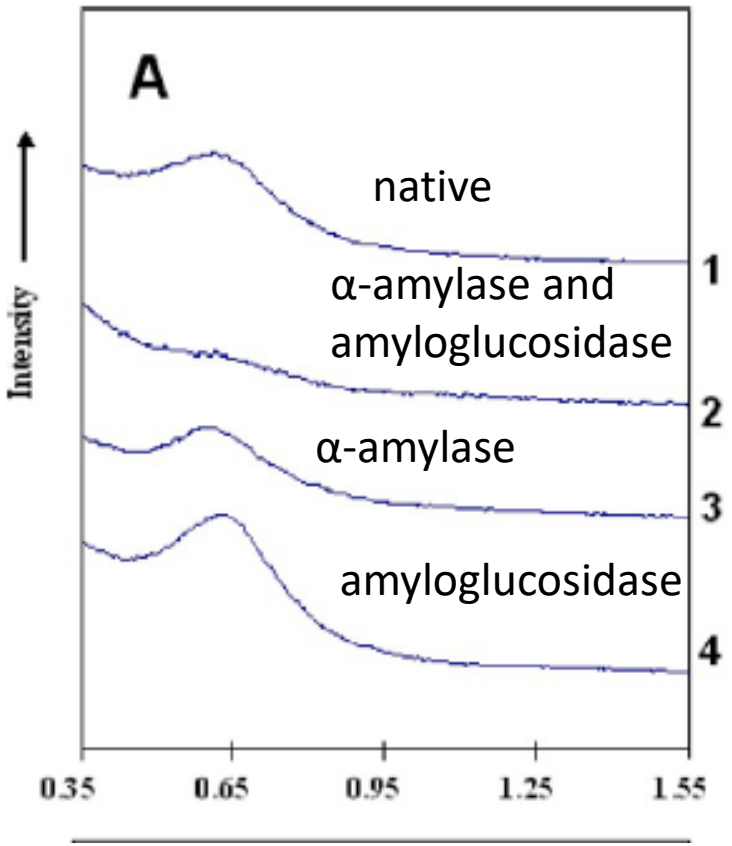
Article

pubs.acs.org/JAFC

Mechanism and Enzymatic Contribution to In Vitro Test Method of Digestion for Maize Starches Differing in Amylose Content

Lauren R. Brewer, Liming Cai, and Yong-Cheng Shi*

College of Agriculture, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, United States



- 'Before and after' study
- Incubated with enzymes for 120 min (I think)

- 9 nm lamellar peak at q ~ 0.65 (1/nm) attributable to the alternative repeating stacks of amorphous and crystalline lamella in starch granules.
- Enzyme mix synergistic in degrading amorphous and crystalline regions (based also on e.g. HPLC analysis)

Structure from carbohydrates – resistant starch

Biomacromolecules **2008**, *9*, 1951–1958

Molecular Rearrangement Of Starch During In Vitro Digestion: Toward A Better Understanding Of Enzyme Resistant Starch Formation In Processed Starches

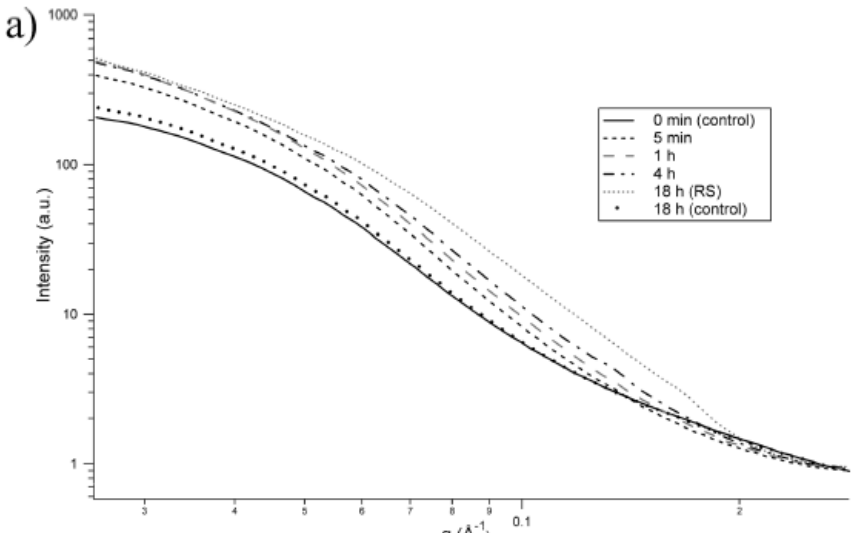
Amparo Lopez-Rubio,*,† Bernadine M. Flanagan,‡ Ashok K. Shrestha,‡,§ Michael J. Gidley,‡ and Elliot P. Gilbert†

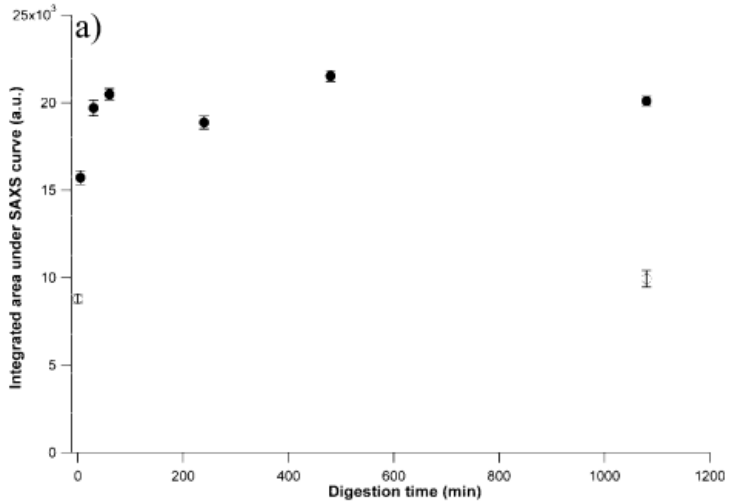
processing conditions as described elsewhere:[12,13] (1) "Mild" conditions → 50% moisture content, 100 °C and 150 s^{-1} shear rate; (2) "Extreme" conditions → 35% moisture content, 140 °C and 750 s^{-1} shear rate.

After processing, the samples were freeze-dried to avoid retrogradation and digested according to the method described in refs 12 and 13.

Isolation of RS Fractions at Different Stages of Digestion. Samples were collected at different times from the digestion solution. Alcohol was added to inactivate the enzymes and the samples were immediately freeze-dried to avoid structural modifications. Two control samples were also prepared in which the extruded samples were kept in the same buffer used for digestion, but without starch hydrolyzing enzymes: Control $t = 0$ (the extruded samples were added into the buffer at 37 °C in which alcohol was added and the samples immediately freeze-dried) and control $t = 18$ h (the extruded starches were kept in the buffer at 37 °C for 18 h in a shaking water bath; after 18 h, alcohol was added and the samples were freeze-dried).

Small Angle X-ray Scattering (SAXS). SAXS measurements were performed on a Bruker Nanostar SAXS camera, as described previously.[13]





Structure from carbohydrates – corn starch

International Journal of Biological Macromolecules 129 (2019) 361–369

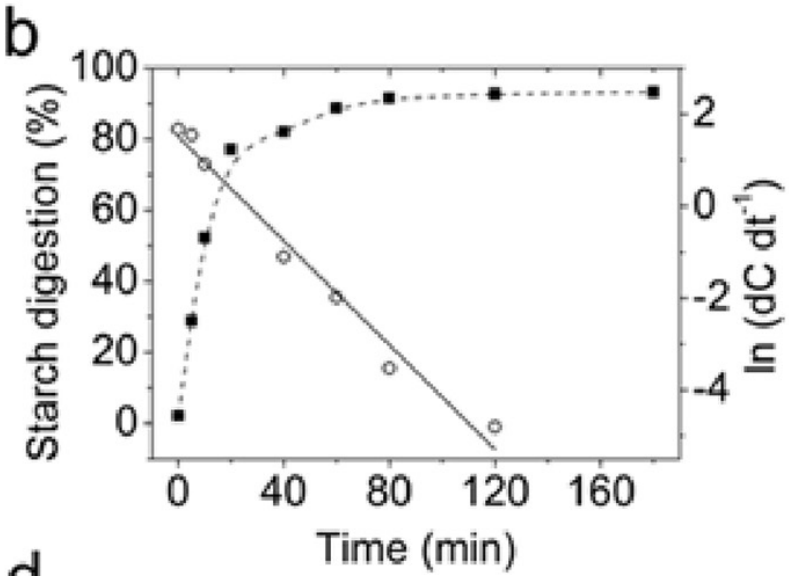
Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Structurally induced modulation of in vitro digestibility of amylopectin corn starch upon esterification with folic acid

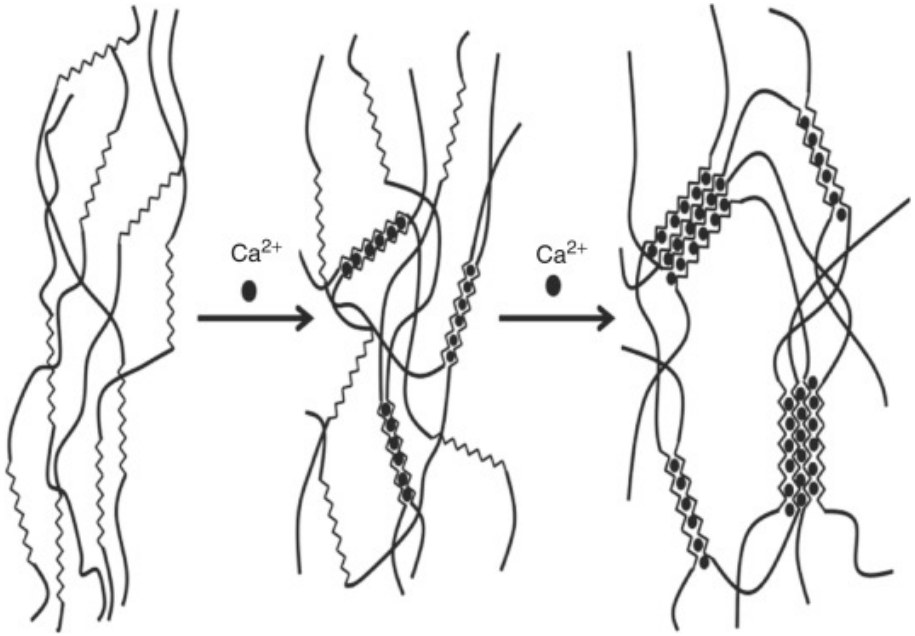
Pallab Kumar Borah [a,b], Michael Rappolt [a], Raj Kumar Duary [b,*], Anwesha Sarkar [a,*]



Time resolved diffraction?

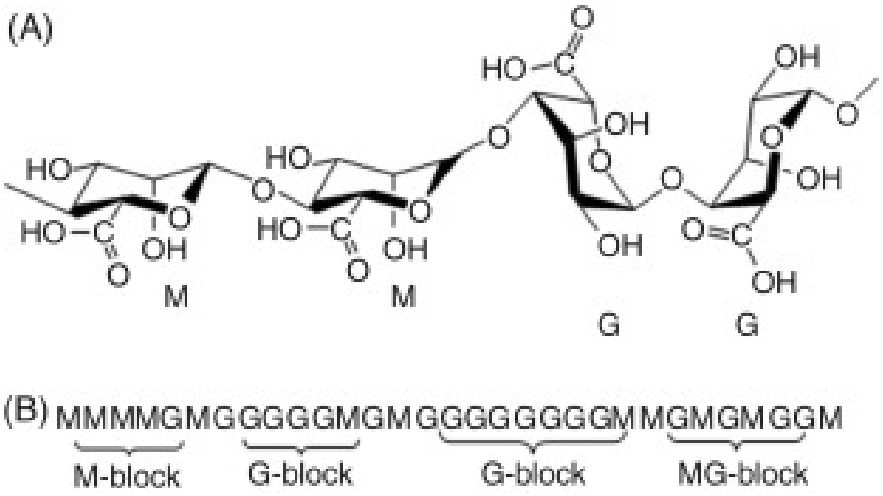
Gastric pH 2.2, with pepsin
Intestinal pH 6.8 pancreatin, amylase, protease, amyloglucosidase and invertase

Structure from carbohydrates - Alginate gels



From brown algae
Calcium cross-linked
Properties depend on M/G ratio
"Egg box" model

(1→4)-linked β-D -mannuronic acid (M) and α-L-gluronic acid (G)



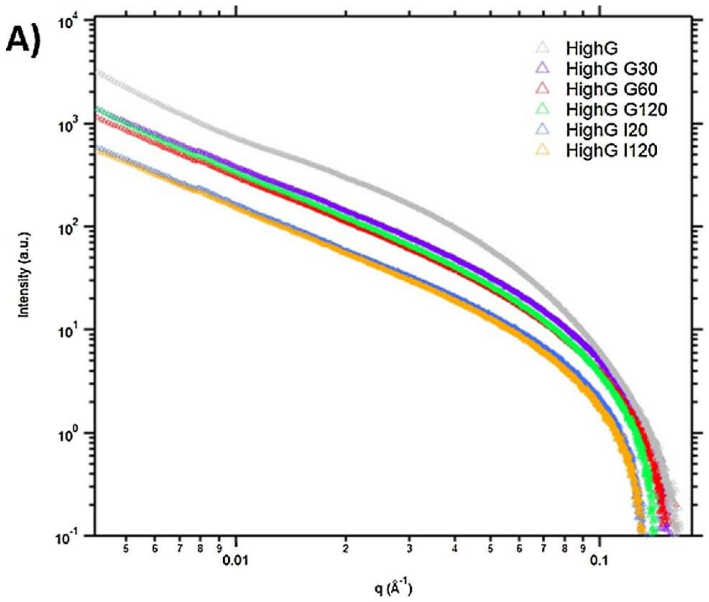
Structure from carbohydrates - Alginate gels

A B S T R A C T

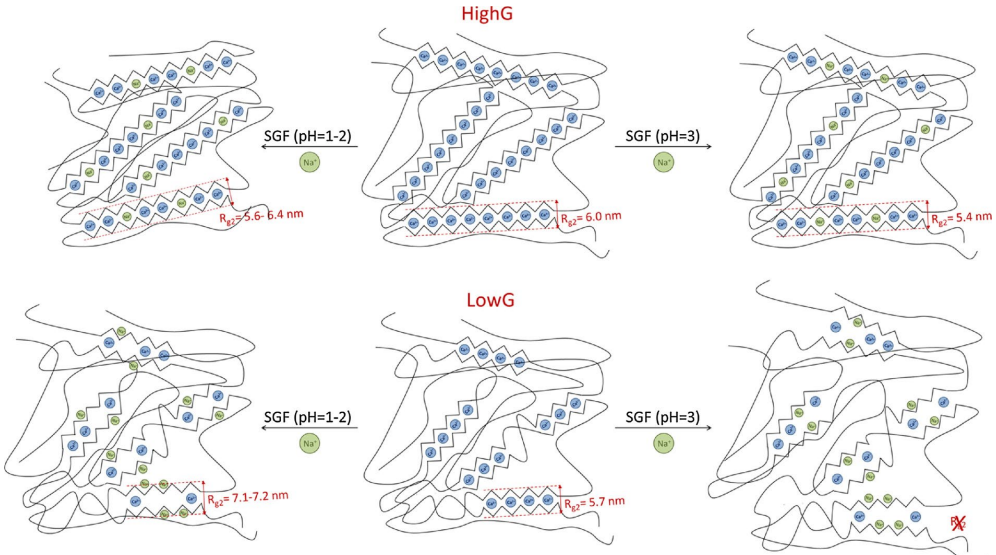
Alginate microcapsules were prepared using three different alginate grades and incubated under simulated digestion conditions

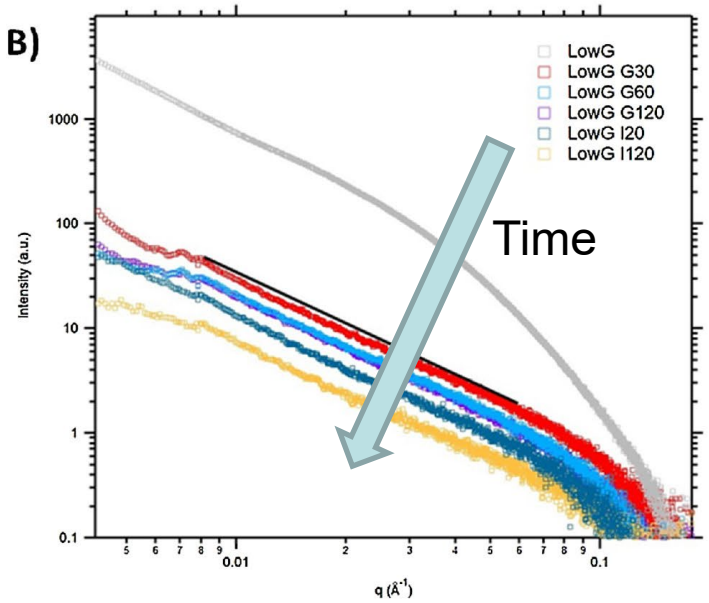
3.3. Impact of in vitro gastrointestinal digestion on the alginate microbeads

In order to assess the micro- and nanostructural changes undergone by the alginate microcapsules upon digestion, these were subjected to simulated gastrointestinal conditions, as described in the standardised static *in vitro* digestion method, developed within the INFOGEST international network (Brodkorb et al., 2019; Minekus et al., 2014). Accordingly, the microcapsules were incubated in simulated gastric fluid (SGF) at a constant pH of 3, and simulated intestinal fluid (SIF, pH = 7), consecutively, at 37 °C for 2 h periods. However, no digestive enzymes or bile were added in this study, since their presence could give rise to the appearance of scattering features, interfering with the



Ca^{2+} apparently displaced by Na^{+}





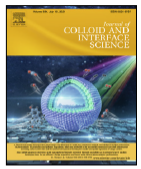
Structure from proteins

Journal of Colloid and Interface Science 594 (2021) 561–574

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

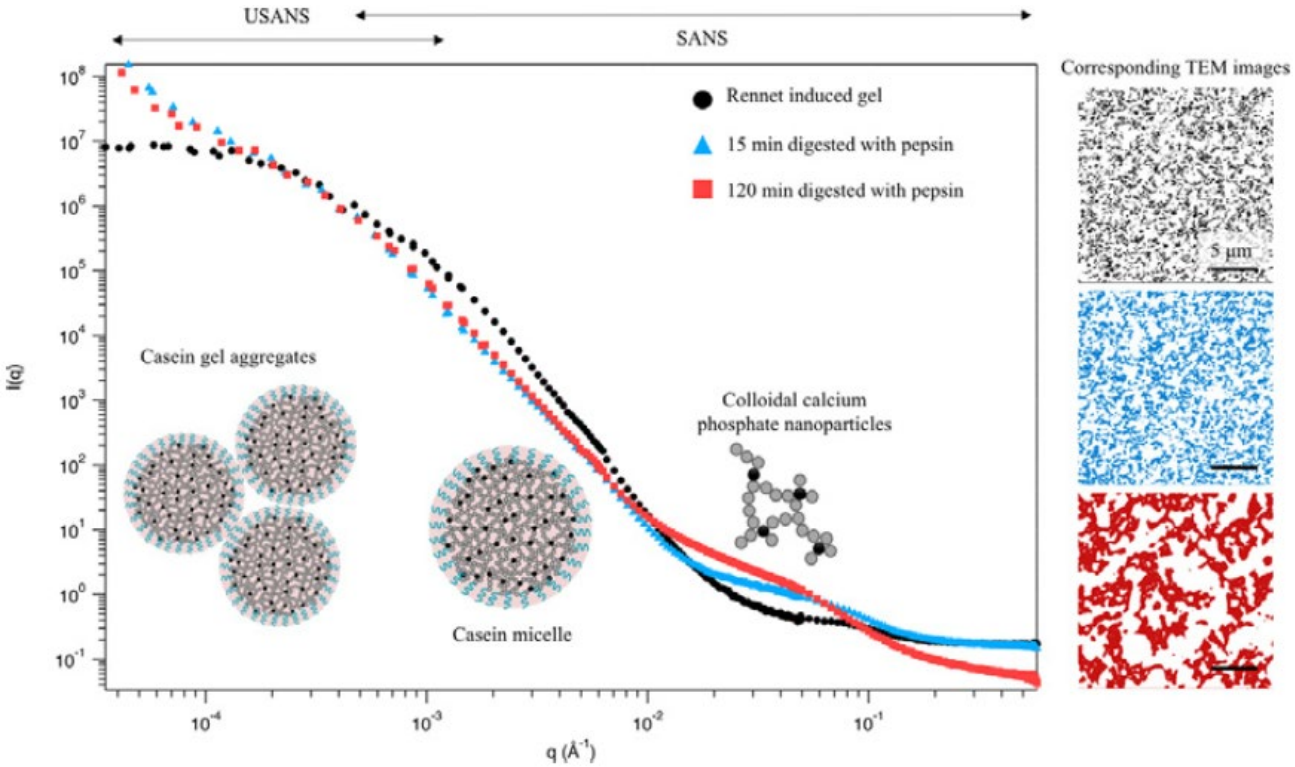


Regular Article

Investigating casein gel structure during gastric digestion using ultra-small and small-angle neutron scattering

Meltem Bayrak [a,b,*], Jitendra Mata [c], Jared K. Raynes [a], Mark Greaves [d], Jacinta White [d], Charlotte E. Conn [b], Juliane Floury [e], Amy Logan [a]

Lots of studies using scattering on casein micelles
Very few actually during digestion



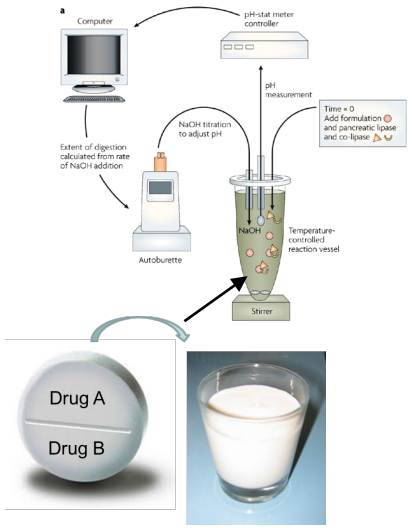
In situ determination of drug disposition

Synchrotron
X- ray source

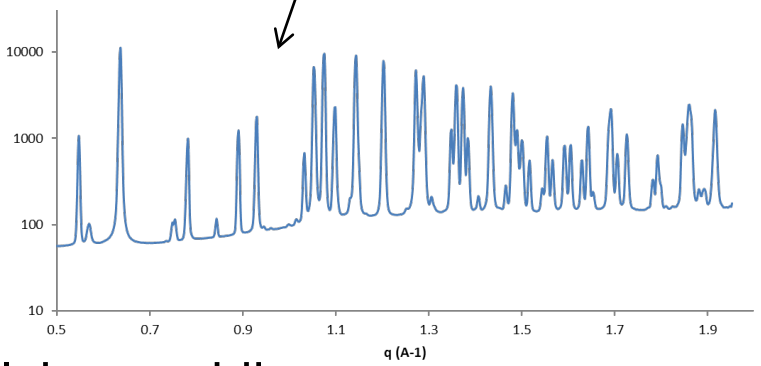
Sample

Detector

integrate



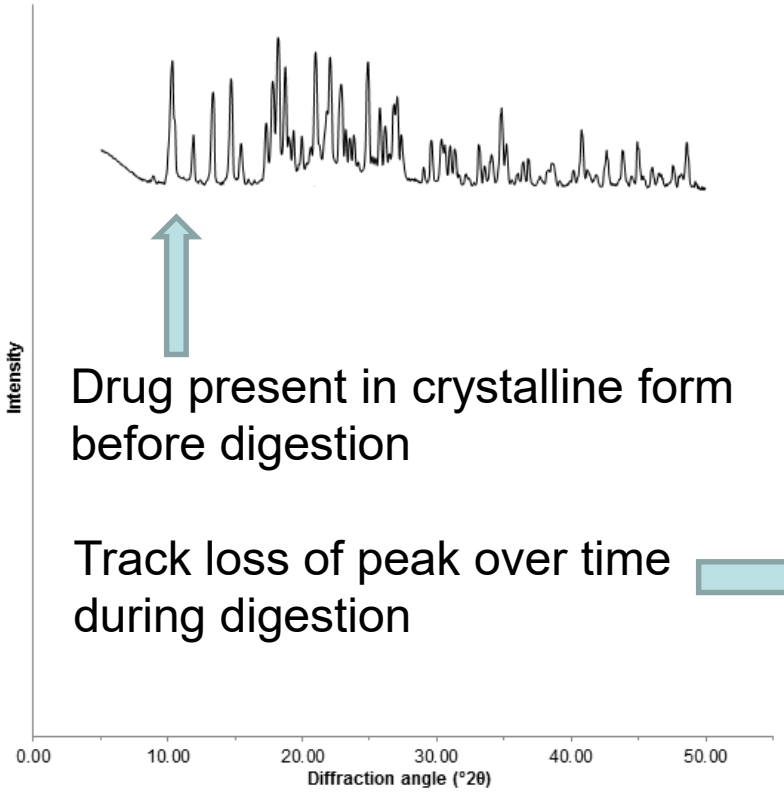
'Measure' how much drug in suspension using intensity of diffraction peaks



- 'Sees' crystalline drug through lipid emulsion avoiding 'separate and sample' difficulties esp. with milk

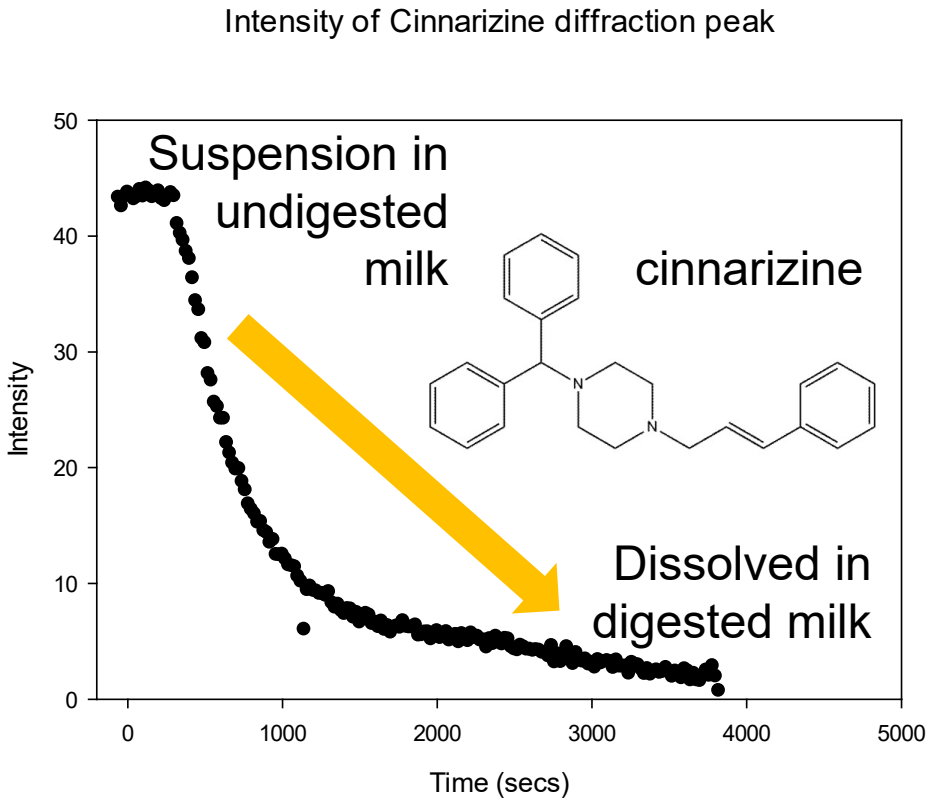
1. Appearance of peaks = precipitation
2. Disappearance = solubilisation/amorphization
3. Change = polymorphic transformation

Real-time solubilization of drug during digestion of milk



Drug present in crystalline form before digestion

Track loss of peak over time during digestion



- Drug has low solubility in milk – drug from disintegrating tablet would not dissolve in primarily milk medium
- Drug is completely soluble in ‘digested’ milk
- Correlates with oral bioavailability

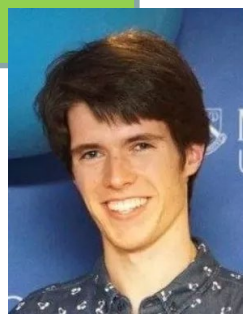
Take home points

- Structure is important – especially during and post-digestion
- Scattering can yield new understanding in food and other soft matter in physiological conditions especially using synchrotron source (but beware need for background subtraction)
- There is a paucity of kinetic data – get out there!

Mimics and fortifiers

Lipid self assembly during digestion

Drug delivery



Why? Evolution, immunological, coincidence

Some practicalities

1. Beware radiation damage (in any *in situ* kinetic experiment)
2. Think ahead about need for background subtraction. Flow through is good but not bullet proof and often not ‘real’ background.
3. Autosampler coupled with flowthrough probably most ideal
4. Consider physiological reality with respect to complexity (which can make 2. difficult), volumes, and also time. Enzymes are usually extracts = high and variable background which in turn makes modelling difficult….
5. Imagination gets you a long way, talking to your beamline staff beforehand gets you even further.

Acknowledgements

Gisela Ramirez

Anna Pham

Syaza Binte Abu Bakar

@group_boyd

nonlamellar.com

Australian Synchrotron

Turning bright ideas into brilliant outcomes

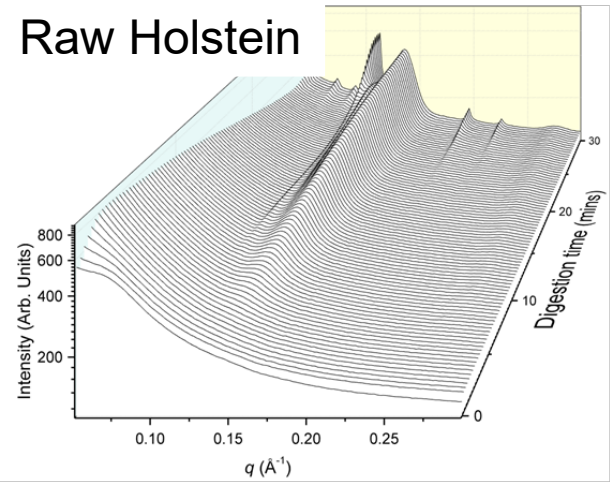
Australian Government

Australian Research Council

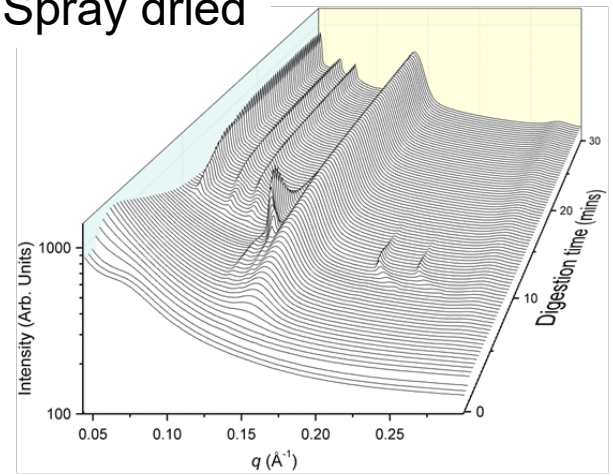
BILL & MELINDA GATES *foundation*

What can we do to milk to upset its behaviour?

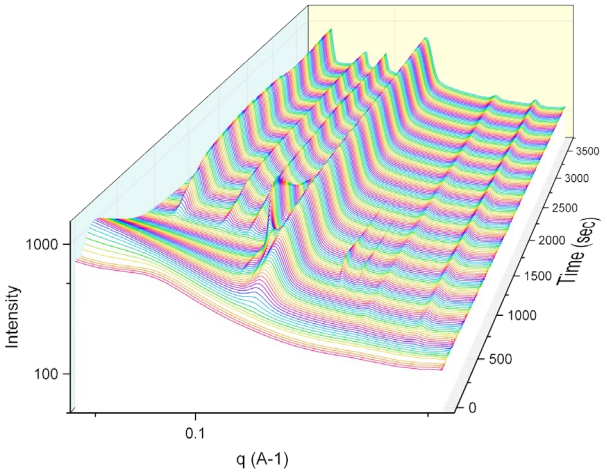
Raw Holstein



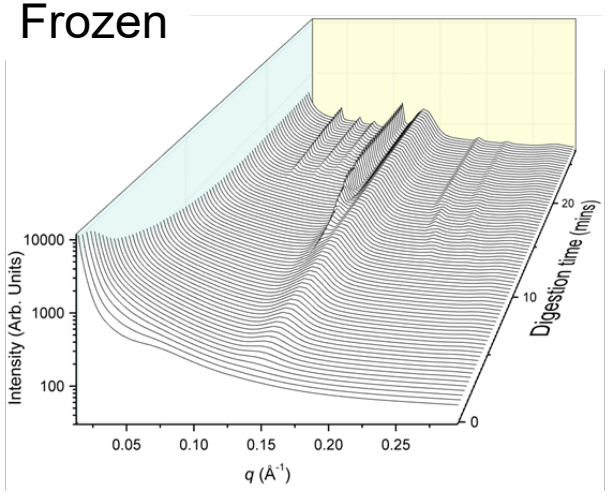
Spray dried



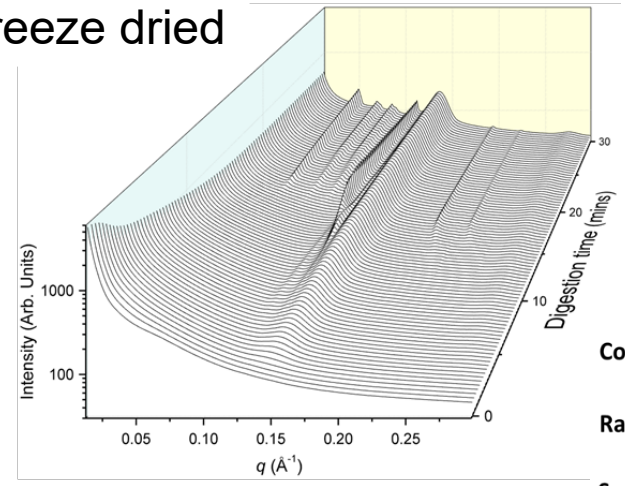
Commercial



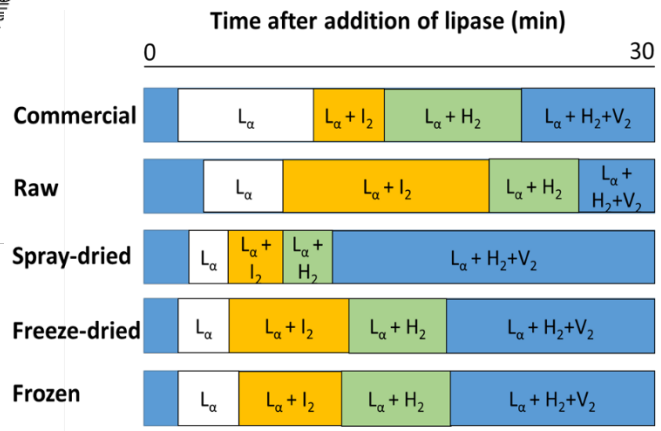
Frozen



Freeze dried

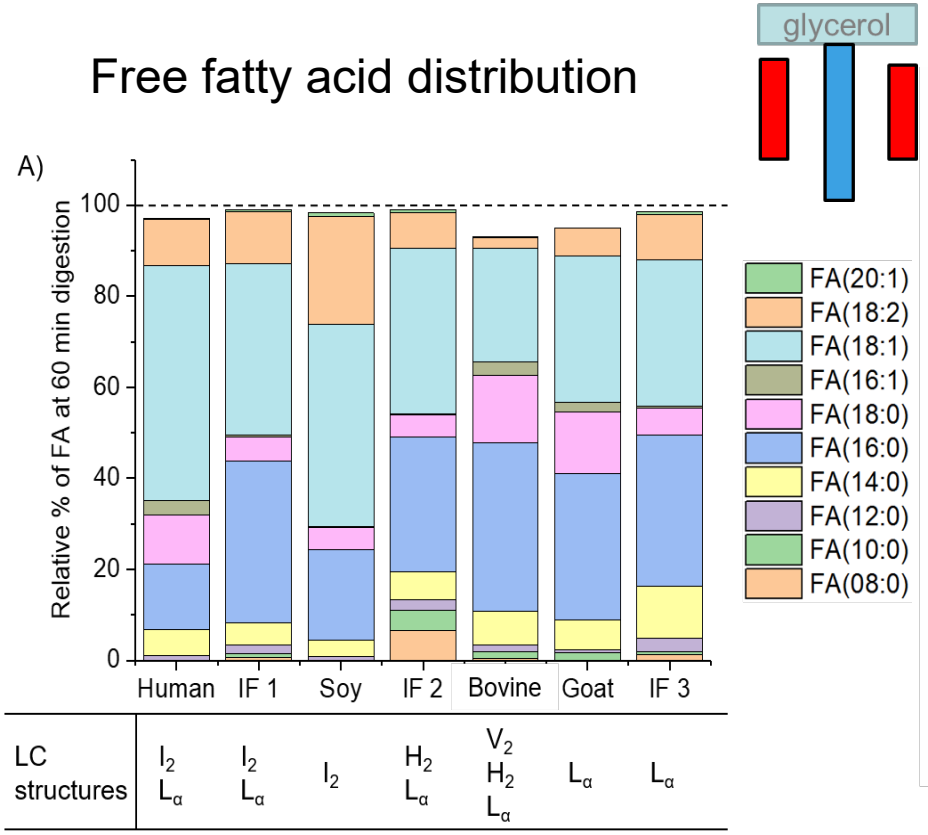


- It's reasonably difficult!

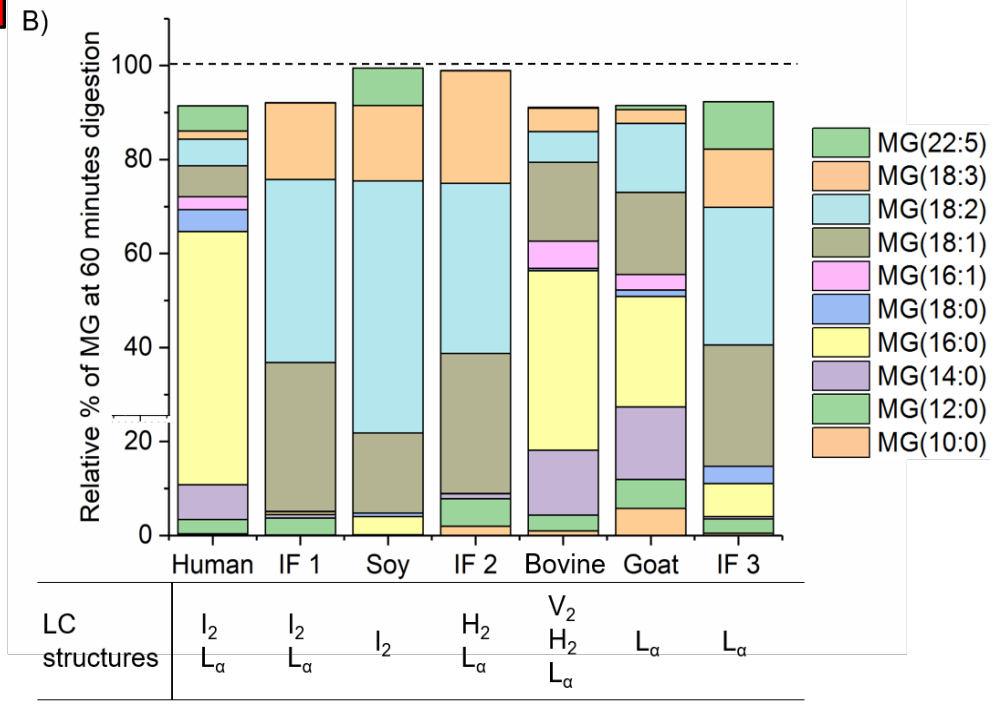


Lipid distribution after digestion complete

Free fatty acid distribution



Monoglyceride distribution

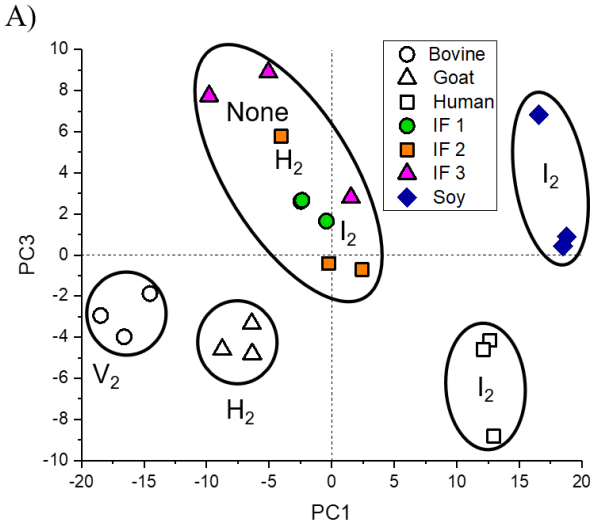


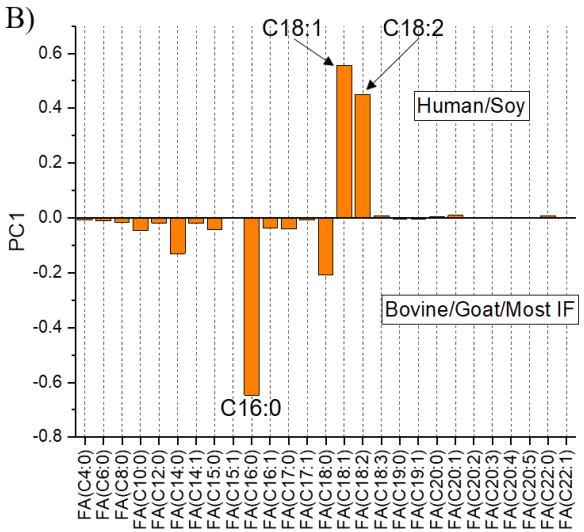
- Monopalmitin much more prevalent in mammal milks than others
- Free fatty acids not as variable
- Infant formulas more greatly resemble Soy (+ other vegetable oils) than milk

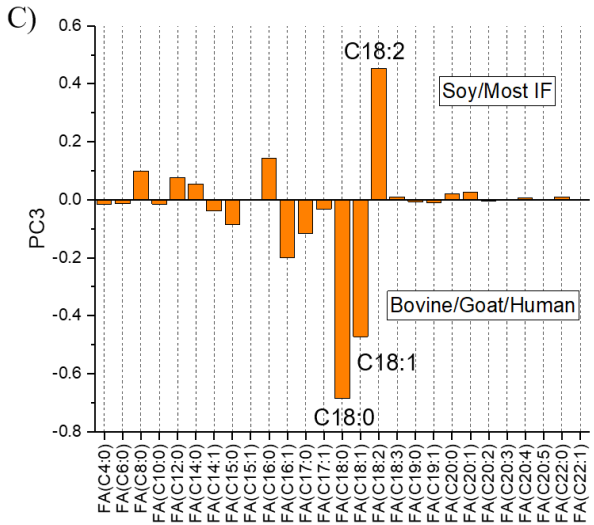
Pham et al. ACS Appl. Bio Mater. 2020, 3, 3087−3098

Lipid distribution after digestion complete

Principal component analysis for fatty acids





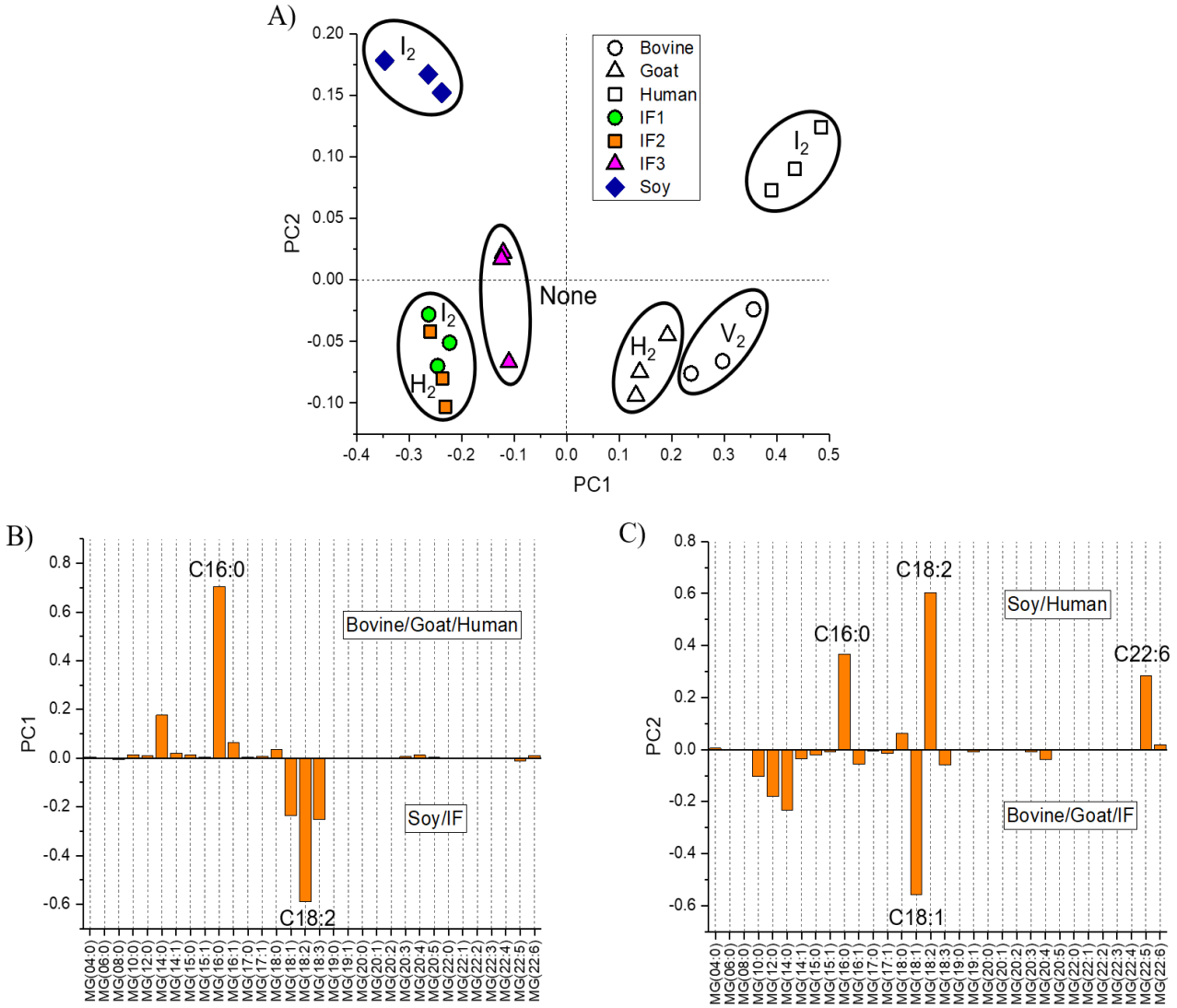


- Primary differences are driven by high oleic/linoleic acid, low palmitic acid

Pham et al. ACS Appl. Bio Mater. 2020, 3, 3087−3098

Lipid distribution after digestion complete

Principal component analysis for monoglycerides



- Primary differences are driven by high monopalmitin, low monoolein/linolinolein

Pham et al. ACS Appl. Bio Mater. 2020, 3, 3087−3098