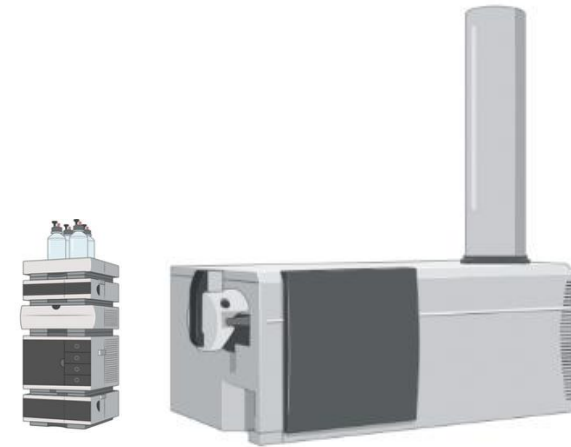


Transforming Dairy Streams 4 health



Daniela Barile

UCDAVIS

FOOD SCIENCE AND TECHNOLOGY





Why caring about
bioactive
compounds?



Major public health
crises of our time:
obesity, diabetes
metabolic syndrome

Threats to life span are
declining.
Yet...threats to life quality
are skyrocketing!



Discovering milk's paradox



UC Davis Milk Processing Lab



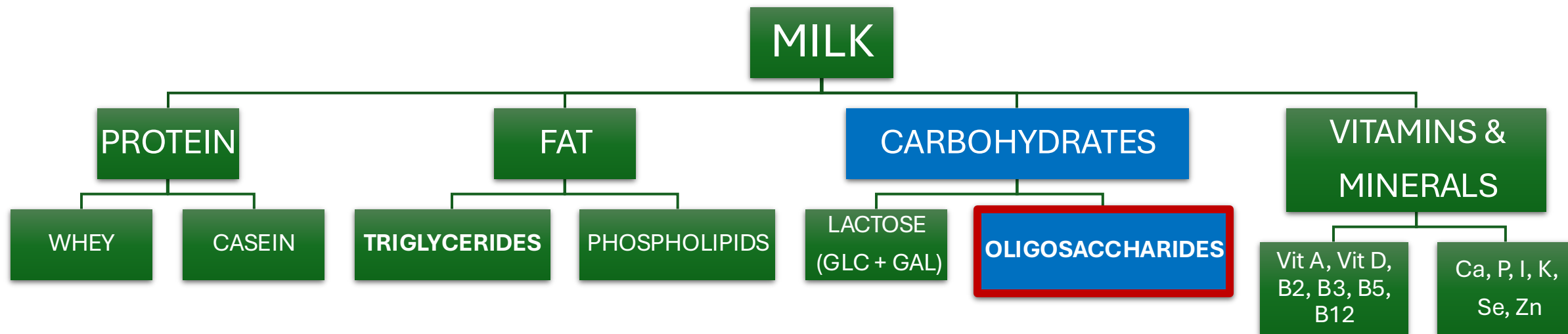
Virtual tour

Foodscience.ucdavis.edu/MPL

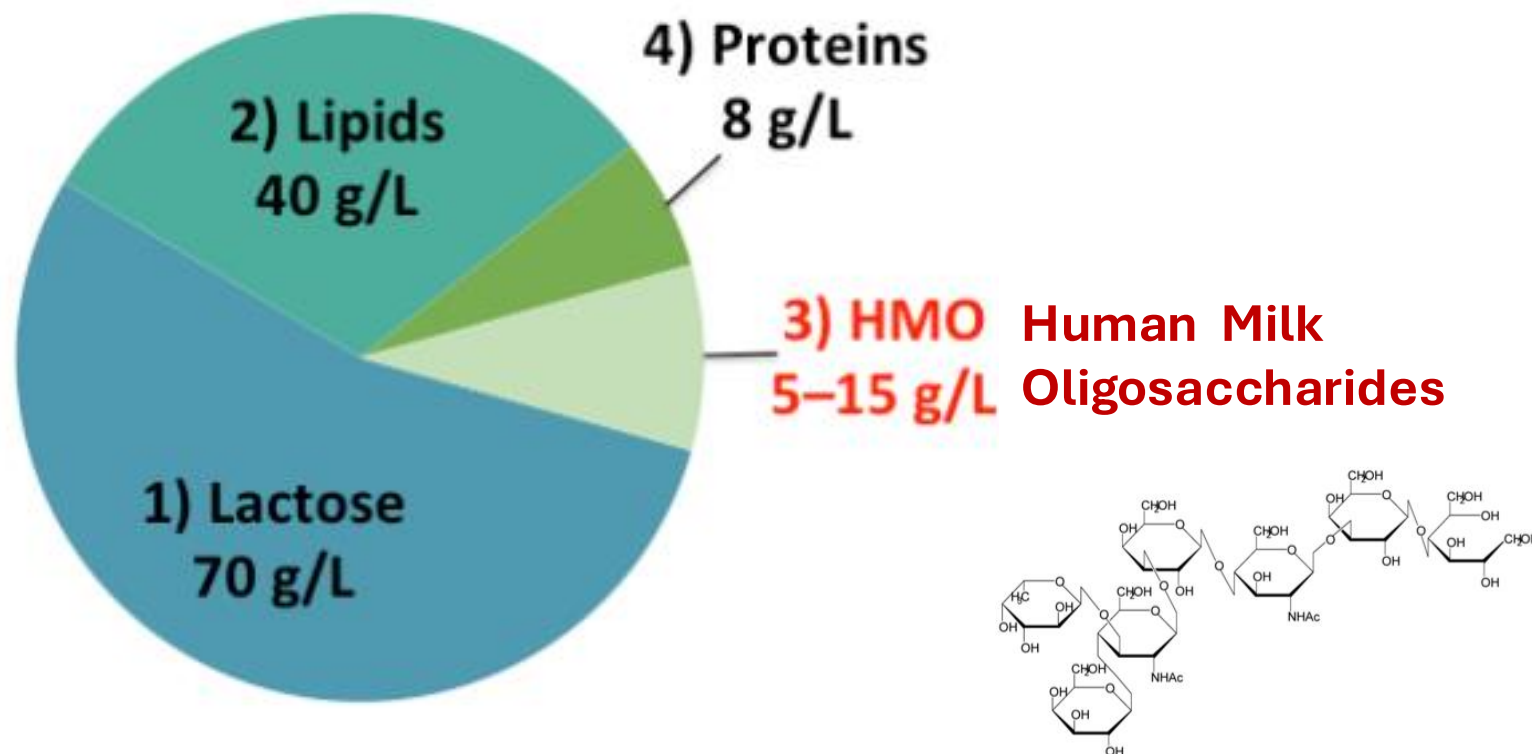
youtube.com/MPL

Take milk apart and discover the **bioactivity** of low-abundant yet **powerful** components.
Improve **human health** and dairy industry **sustainability**.
Train future workforce!



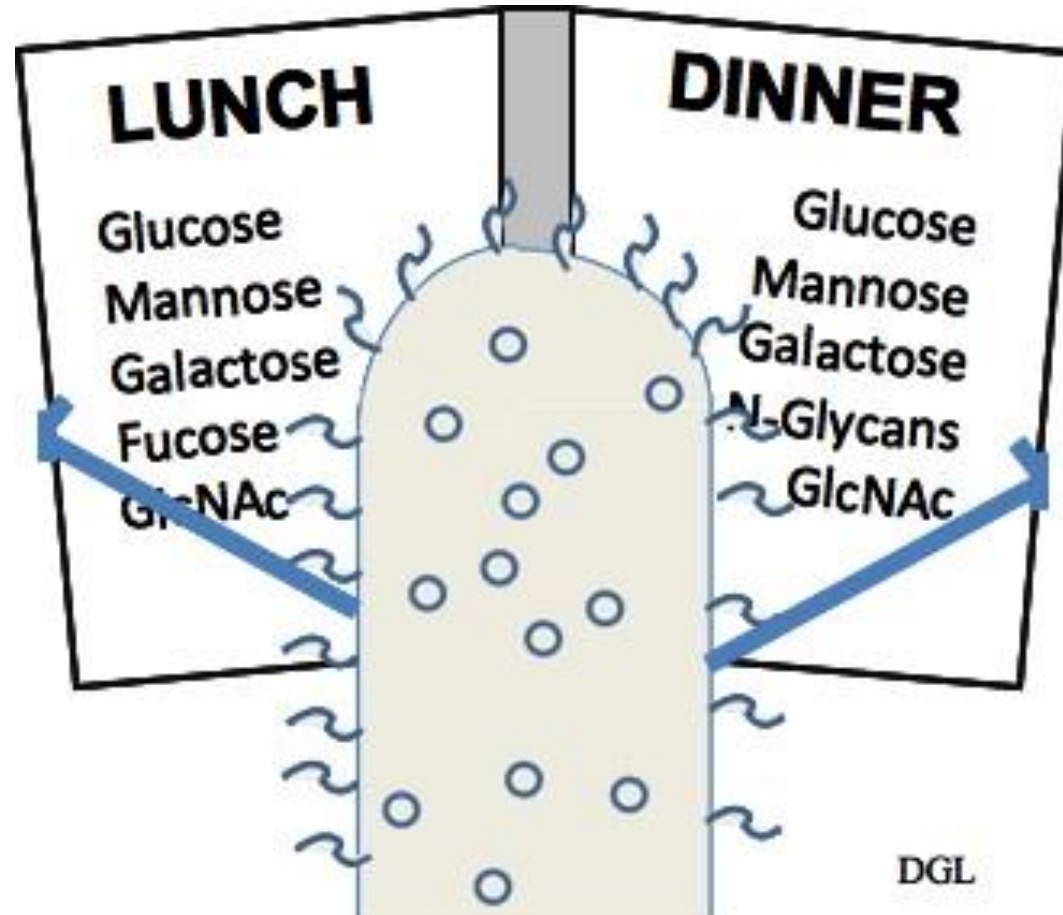


in the past, scientists were puzzled about oligosaccharides presence in mammalian milk



Solving milk paradox: oligosaccharides promote Bifidobacteria

Oligosaccharides
are completely
indigestible !



Feed Bacteria that evolved to consume milk sugars



Chemistry unlocks key molecules and their functions

1950

Bifidus Factor. I. A Variant of *Lactobacillus bifidus* Requiring a Special Growth Factor¹

Oligosaccharides of Human Milk

Isolation and Characterization of Three New Disialylfucosyl Hexasaccharides

1976

Human milk oligosaccharides: 130 reasons to breast-feed

2000

Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract¹⁻³

Over 32,000
scientific articles
published

2024

The functional biology of human milk oligosaccharides

Nursing our microbiota: molecular linkages between bifidobacteria and milk oligosaccharides

DA Sela, DA Mills [+ Paperpile](#)

Trends in microbiology 18 (7), 298-307

Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition

MR Charbonneau, D O'Donnell, LV Blanton, SM Totten, JCC Davis, ... [+ Paperpile](#)

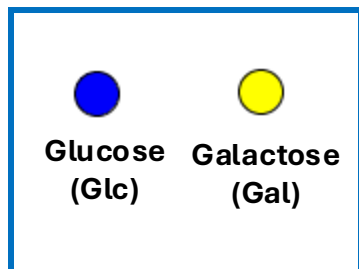
Cell 164 (5), 859-871



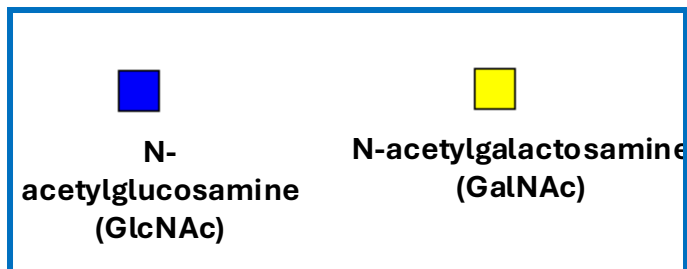
Lactose

Oligosaccharides / glycans on proteins

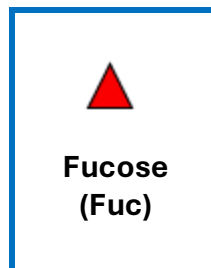
Building Block Monosaccharides



Hex



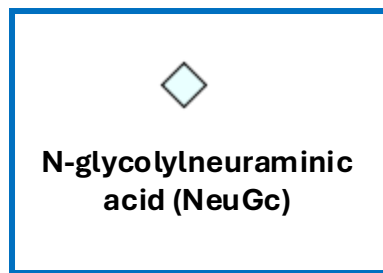
HexNA



Fuc



NeuAc



NeuGc (bovine)

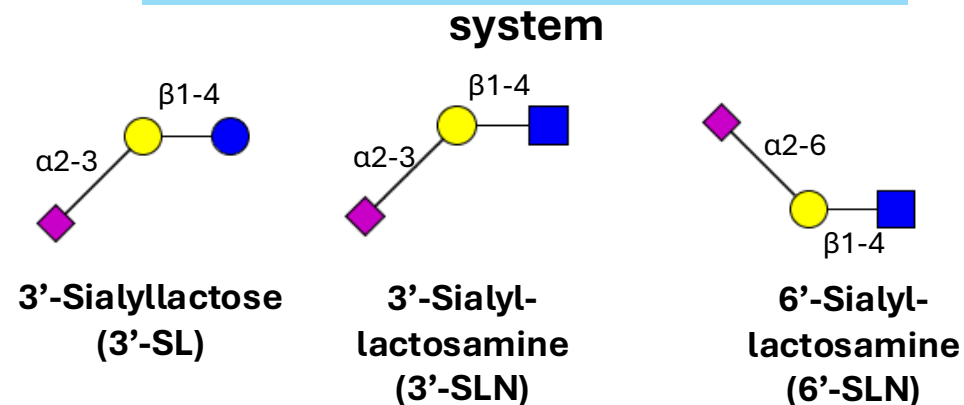
Difficult to study!

- Complex nomenclature
- Lack of automated data analysis

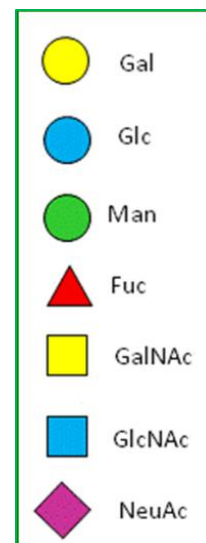
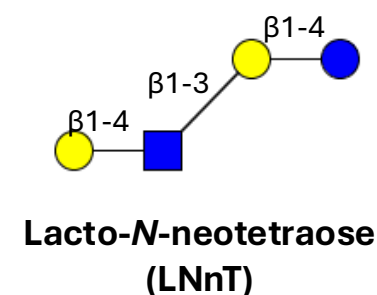
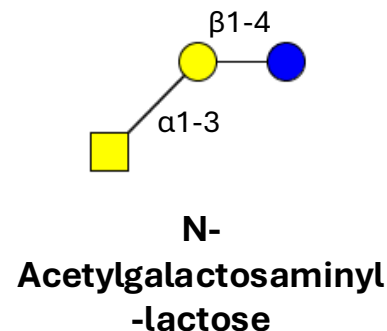
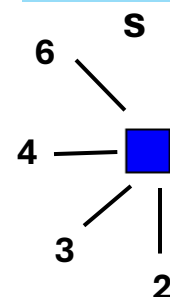
They are indigestible

Hundreds of complicated structures

Examples of simple oligosaccharides & coding system



Linkage

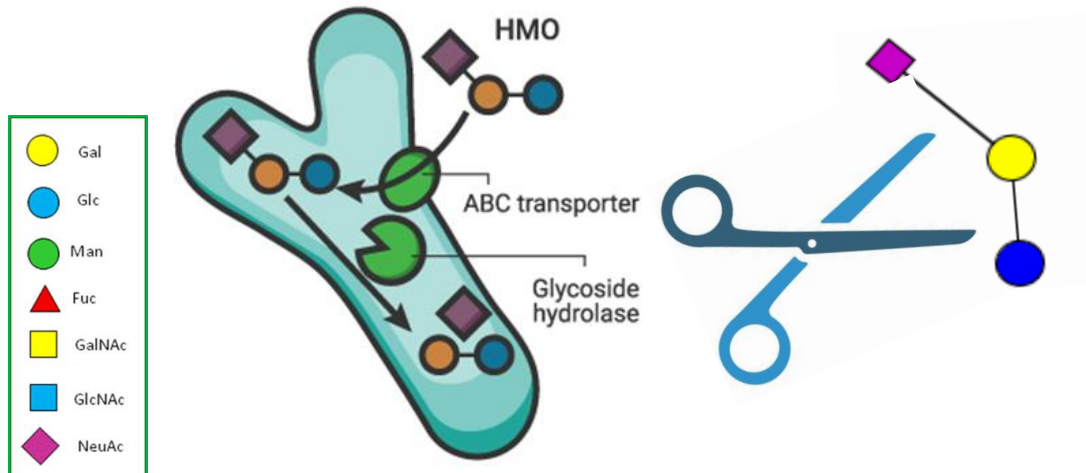


Demonstrated benefits / mechanisms

- Protect from pathogens
- Serve as food **only** for beneficial gut bacteria
- Have diverse structures (prebiotic selectivity)
- Help brain development



Prolacta
BIOSCIENCE



Randomized Controlled Trial > J Pediatr Gastroenterol Nutr. 2014 Mar;58(3):352-60.
doi: 10.1097/MPG.0000000000000211.

Prebiotic oligosaccharides in premature infants

Mark A Underwood¹, Karen M Kalanetra, Nicholas A Bokulich, Majid Mirmiran, Daniela Barile, Daniel J Tancredi, J Bruce German, Carlito B Lebrilla, David A Mills

- ✓ Bifidobacteria have specific enzymes
- ✓ Produce anti-inflammatory molecules

ANNUAL REVIEW OF ANIMAL BIOSCIENCES Volume 3, 2015

Review Article | Free

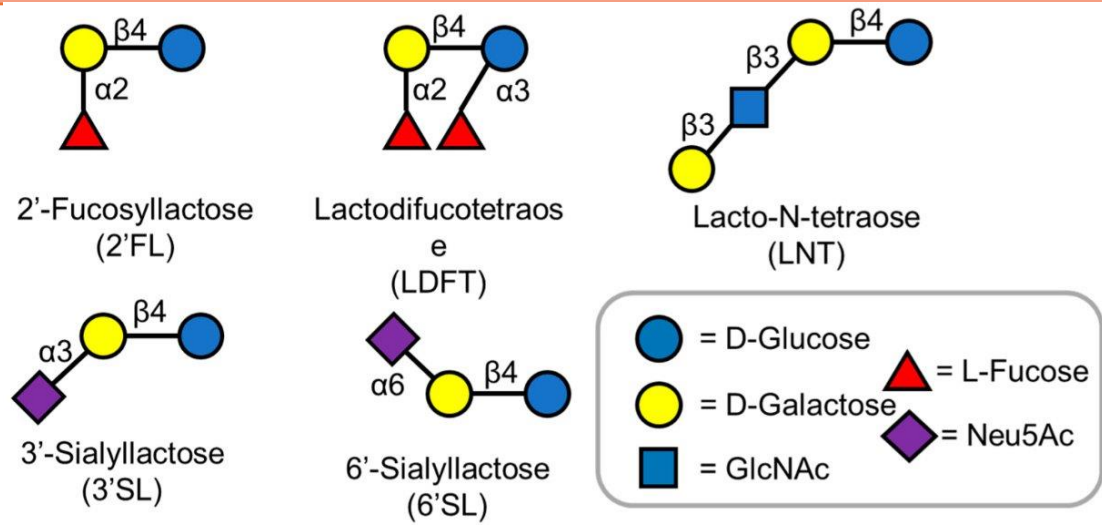
The Impact of the Milk Glycobiome on the Neonate Gut Microbiota

Alline R. Pacheco^{2,3}, Daniela Barile^{2,3}, Mark A. Underwood^{2,4}, and David A. Mills^{1,2,3}

Not all prebiotics are created equal

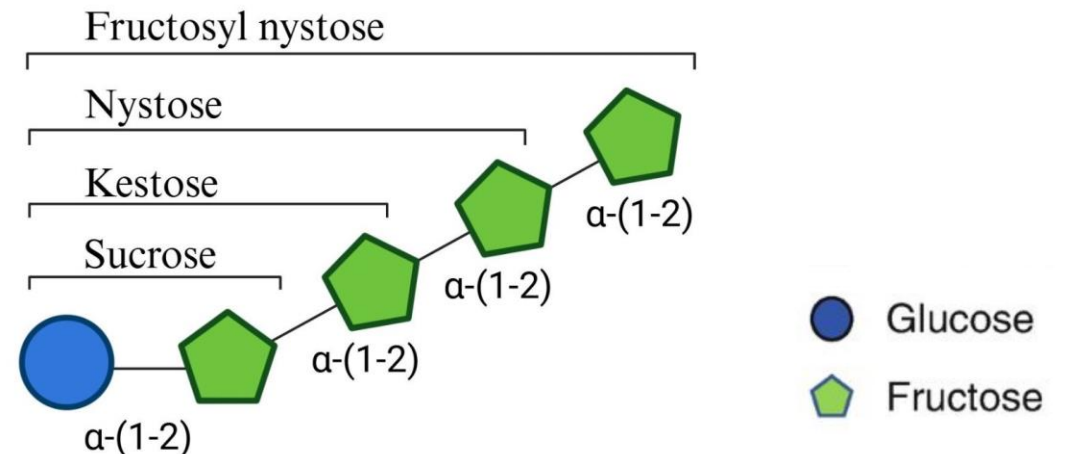
Human milk oligosaccharides (gold standard)

- Promote maturation of the intestine and select a protective microbiota
- Guide establishment of healthy immune system and brain development



Fructooligosaccharides & Inulin

- Ability to promote the growth of a variety of bacteria
- Low structural diversity (same units)
- Common side effects include bloating, cramps and diarrhea



Lessons learned from milk



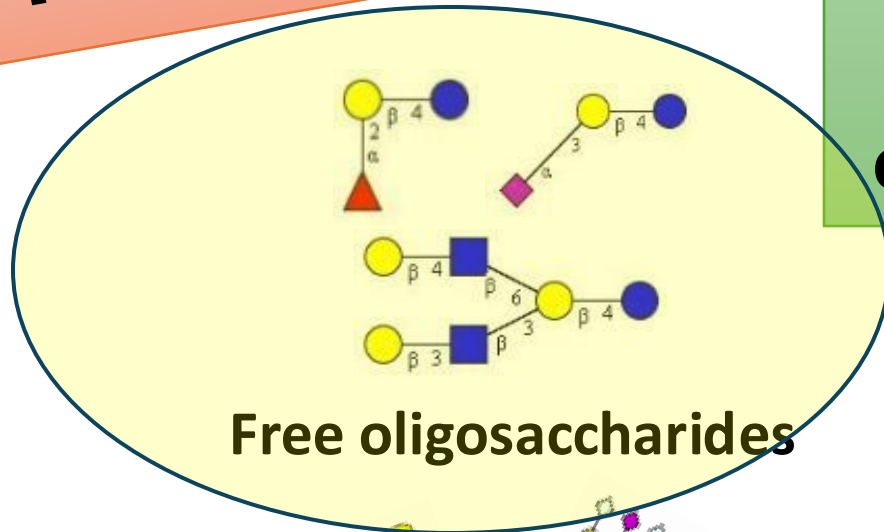
Inoculating the gut with bifidobacteria
(and selectively feeding them with oligosaccharides)
is key to prevent infection, inflammation and more.

Can we translate this knowledge?

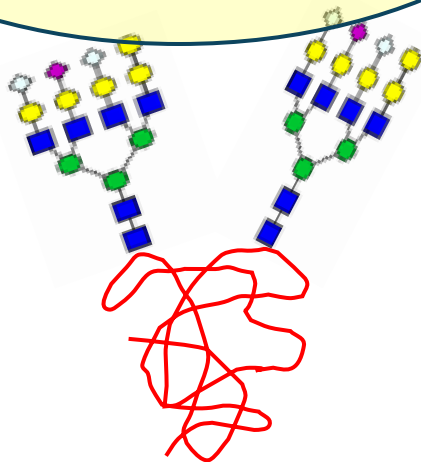


A recent science: Glycomics

comprehensive study of sugars,
free
or attached to other molecules

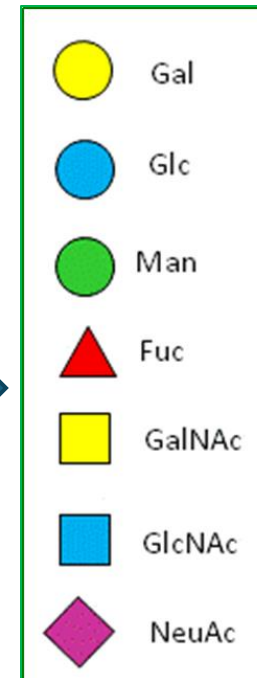


Free oligosaccharides



Protein-linked glycans

Symbolic
nomenclature:
monosaccharides are
denoted by specific
colored geometric
shapes



(Consortium for Functional Glycomics)

Knowledge of structures & concentrations is needed for determining biological functions and formulating new ingredients



NANO-LC Q TOF



HPAE-PAD



MALDI-TOF



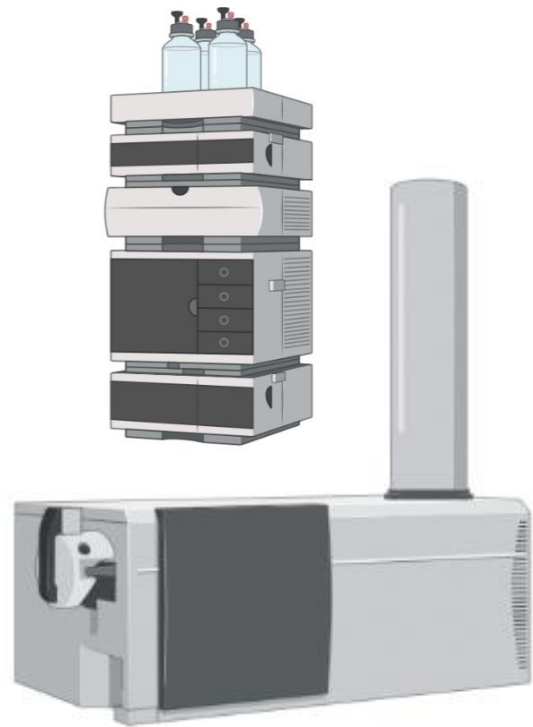
6470A Triple Quad LC-MS

- Multiple analytical techniques are necessary
- High mass accuracy and mass resolution
- Guide processing and ensure purity of ingredients

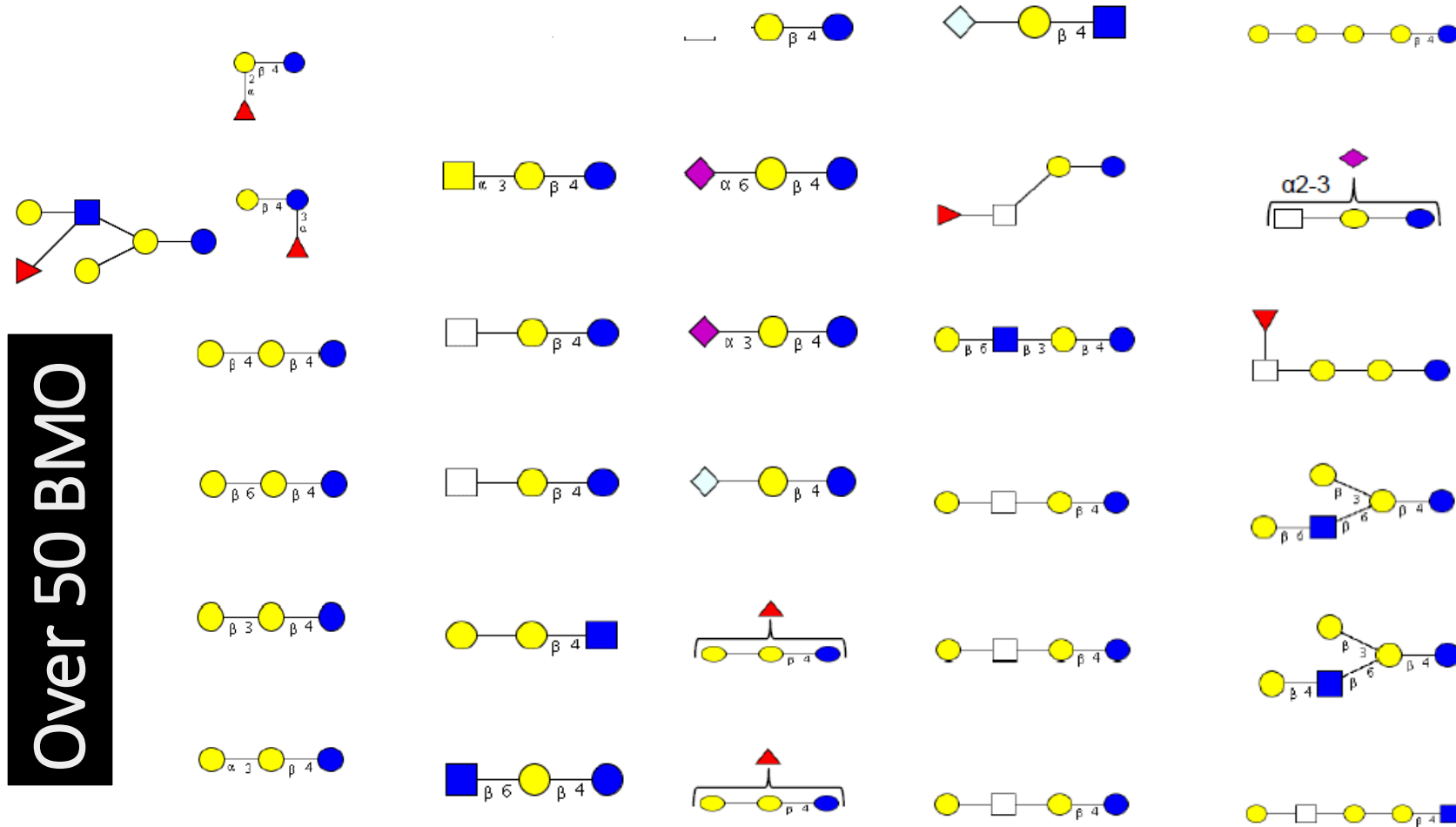
Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures FREE

Danielle L Aldredge¹, Maria R Geronimo, Serenus Hua, Charles C Nwosu, Carlito B Lebrilla, Daniela Barile

Comparative Study > *Glycobiology*. 2013 Jun;23(6):664-76.



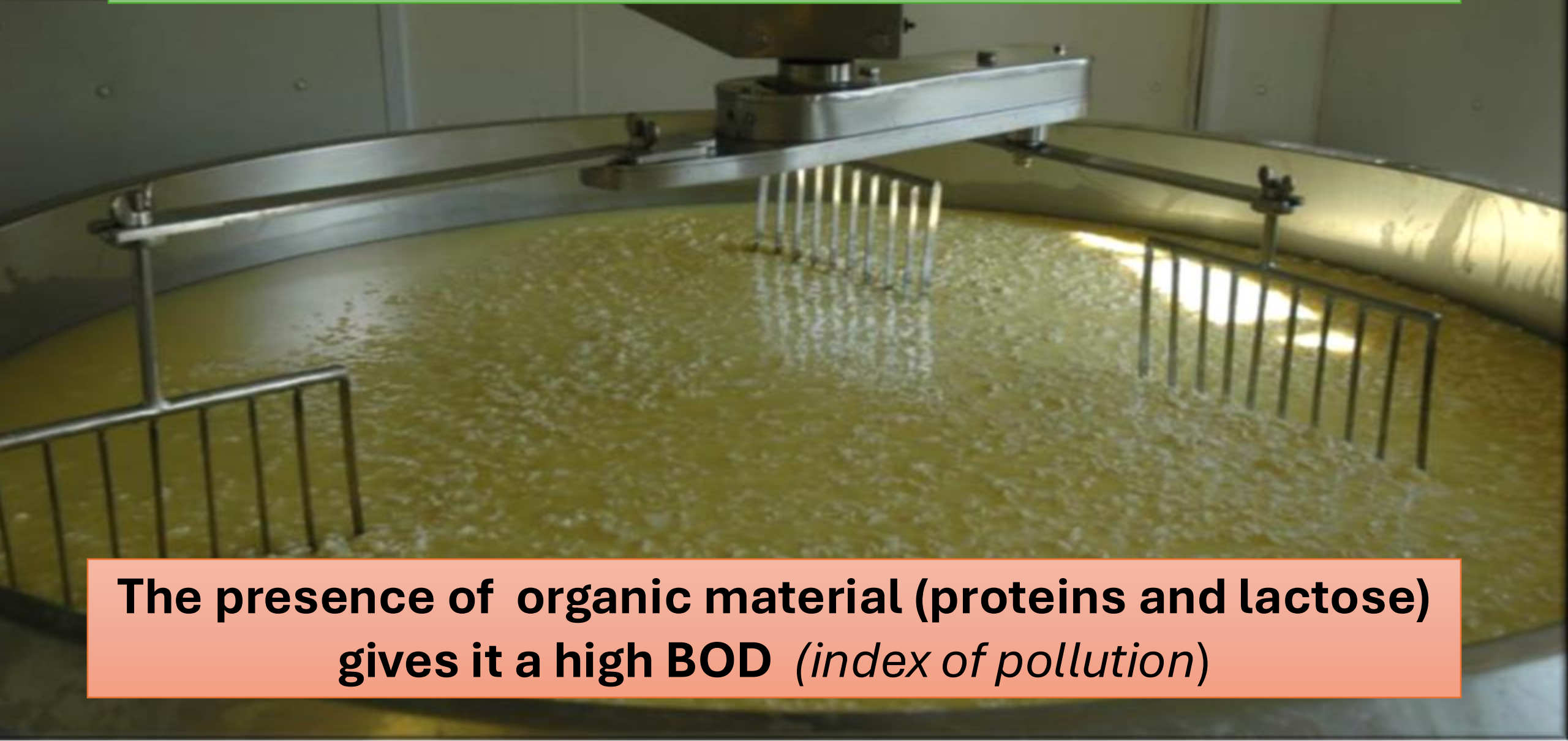
Over 50 BMO



Many structures identical/similar to human milk

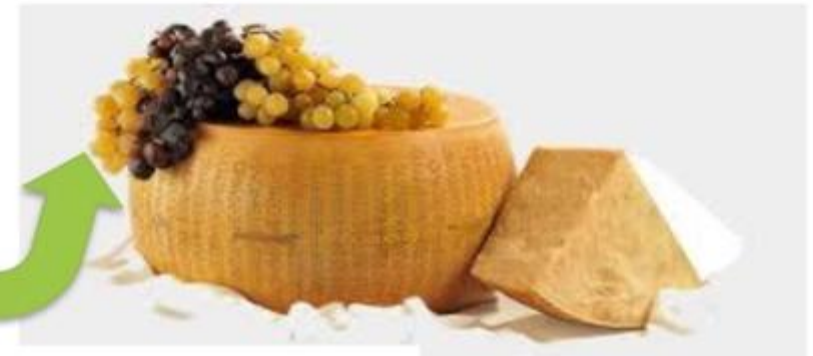
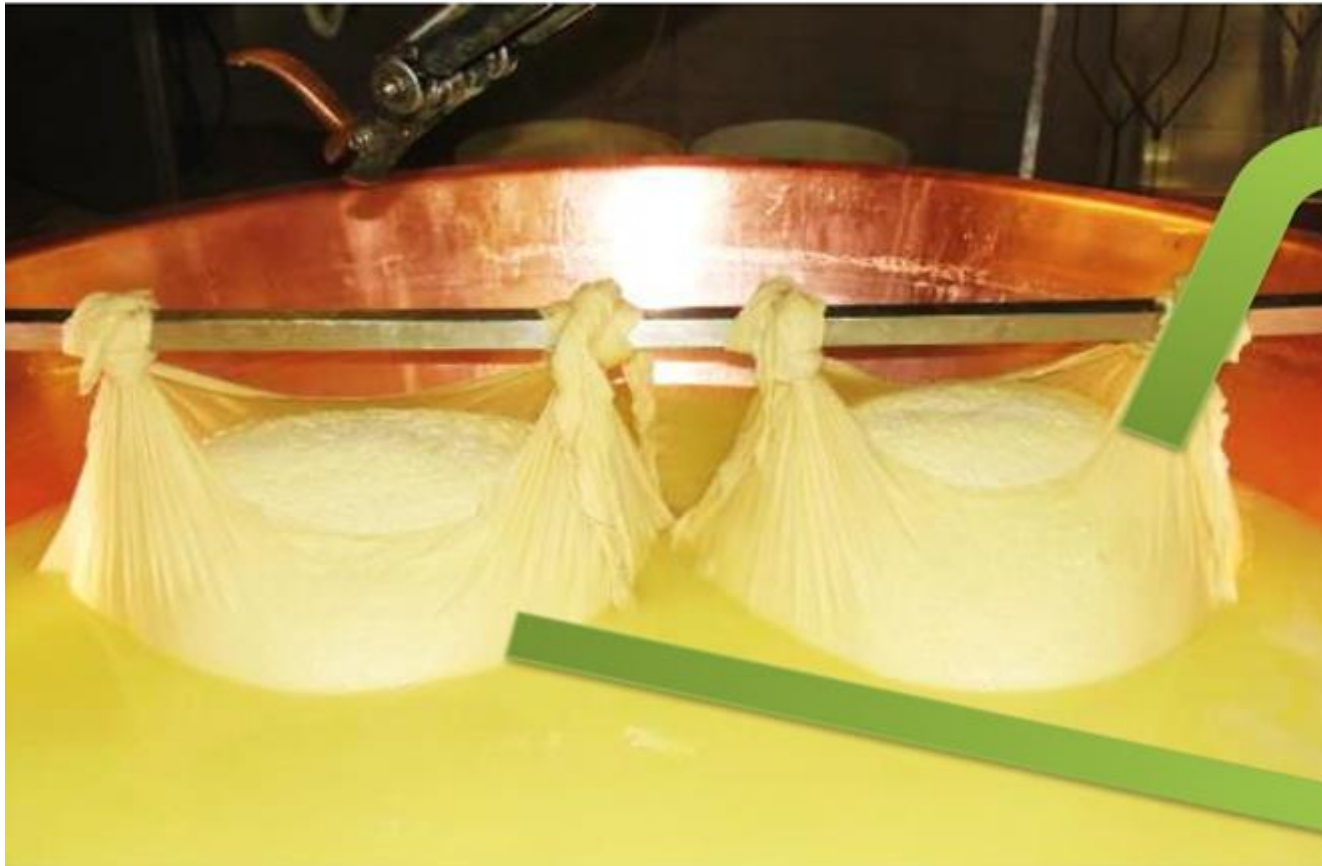


**50 Million lb/day in California.
Still undervalorized**



**The presence of organic material (proteins and lactose)
gives it a high BOD (*index of pollution*)**

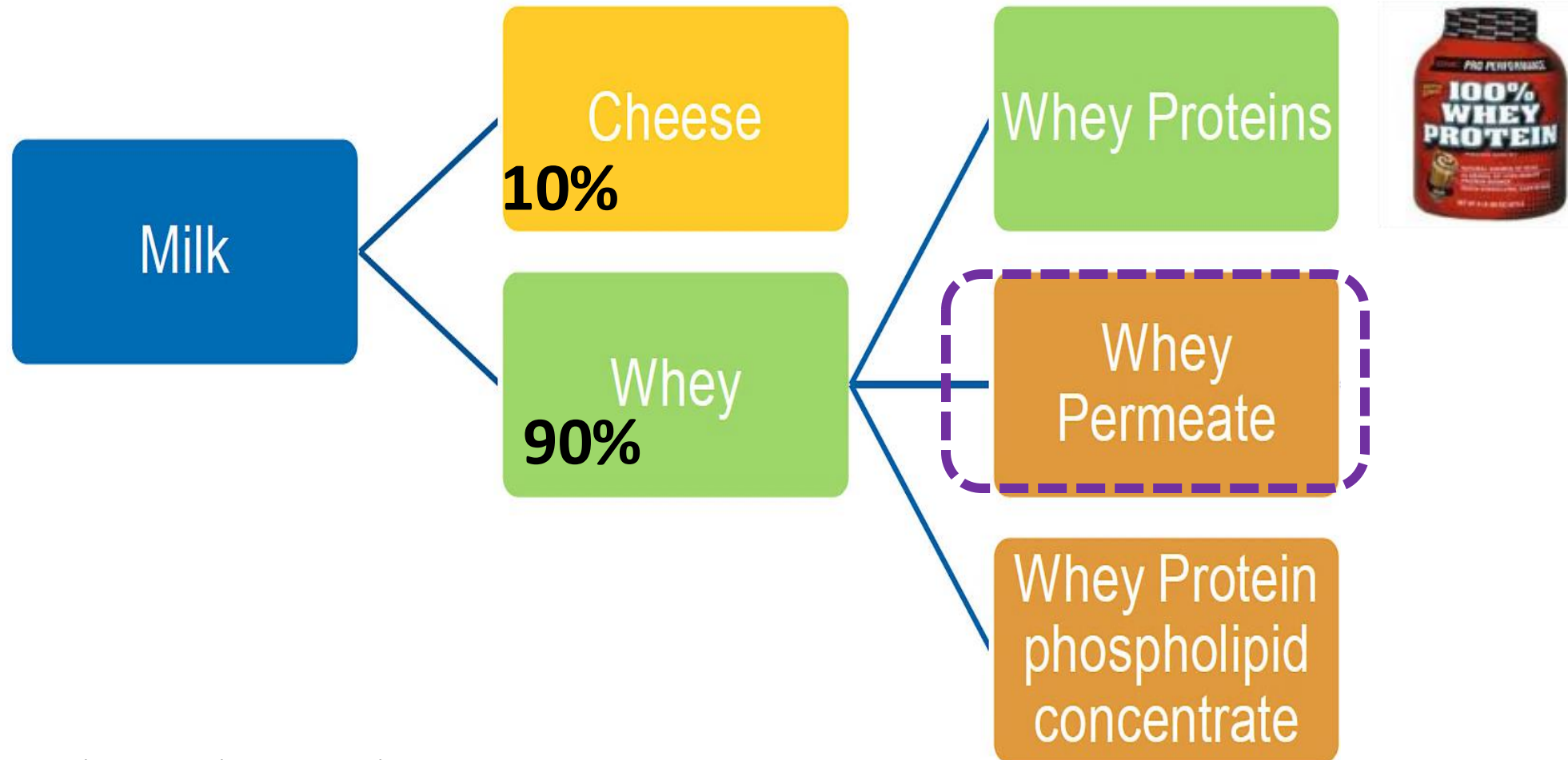
1100 liters of milk needed to
produce 2 wheels of cheese
(~ 100 kg of cheese)
Yield: ~10% cheese; 90% whey



Restructuring
agriculture

Find value in waste
streams
Reduce outputs into
the environment

Looking for oligosaccharides/glycans in whey streams



Investigation oligosaccharides activities in whey permeate

Purify oligosaccharides
from whey permeate
in our pilot plant



UC Davis
MILK PROCESSING LAB
& industry collaborators



Mills' LAB



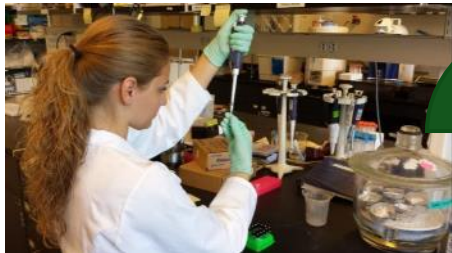
Test commensal bacteria

Glycoprofiling
by mass spec



Animal Models of
metabolic disorder

Reduced Inflammation,
Improved gut barrier,
Reduced weigh gain



Quality control
by mass spec



2016 **Cell** 164 (5), 859-871

2019 **PNAS** 116 (24), 11988-11996



2017 *Am. J of Physiology-Gastrointestinal and liver diseases* 312 (5)

2018 *The Journal of nutritional biochemistry* 57, 246-254



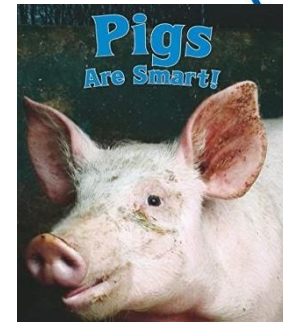
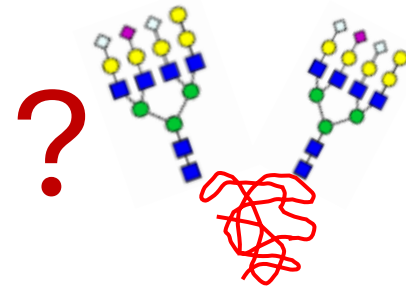
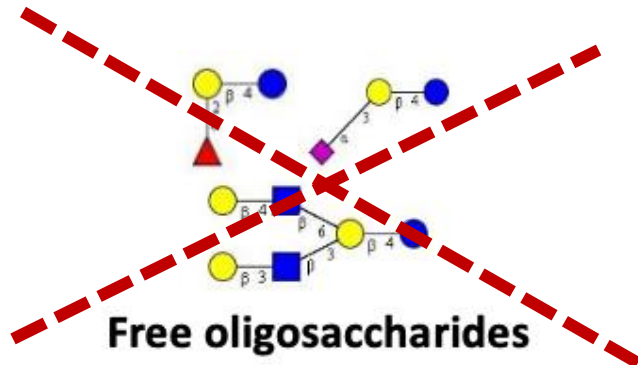
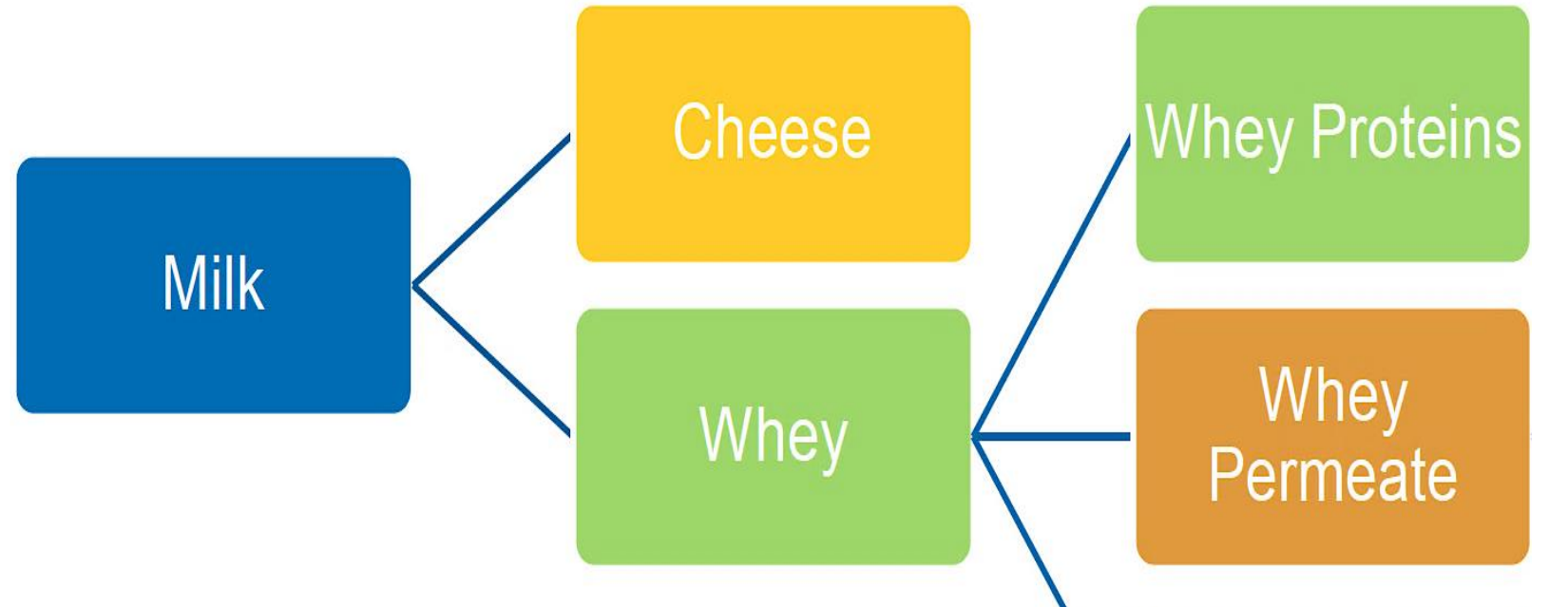
Raybould's
Lab

To attain circular bioeconomy
each sequential stream must be valorized
with minimal energy use



What if we could skip extraction and still get prebiotics?

Looking at available whey streams for protein-linked glycans



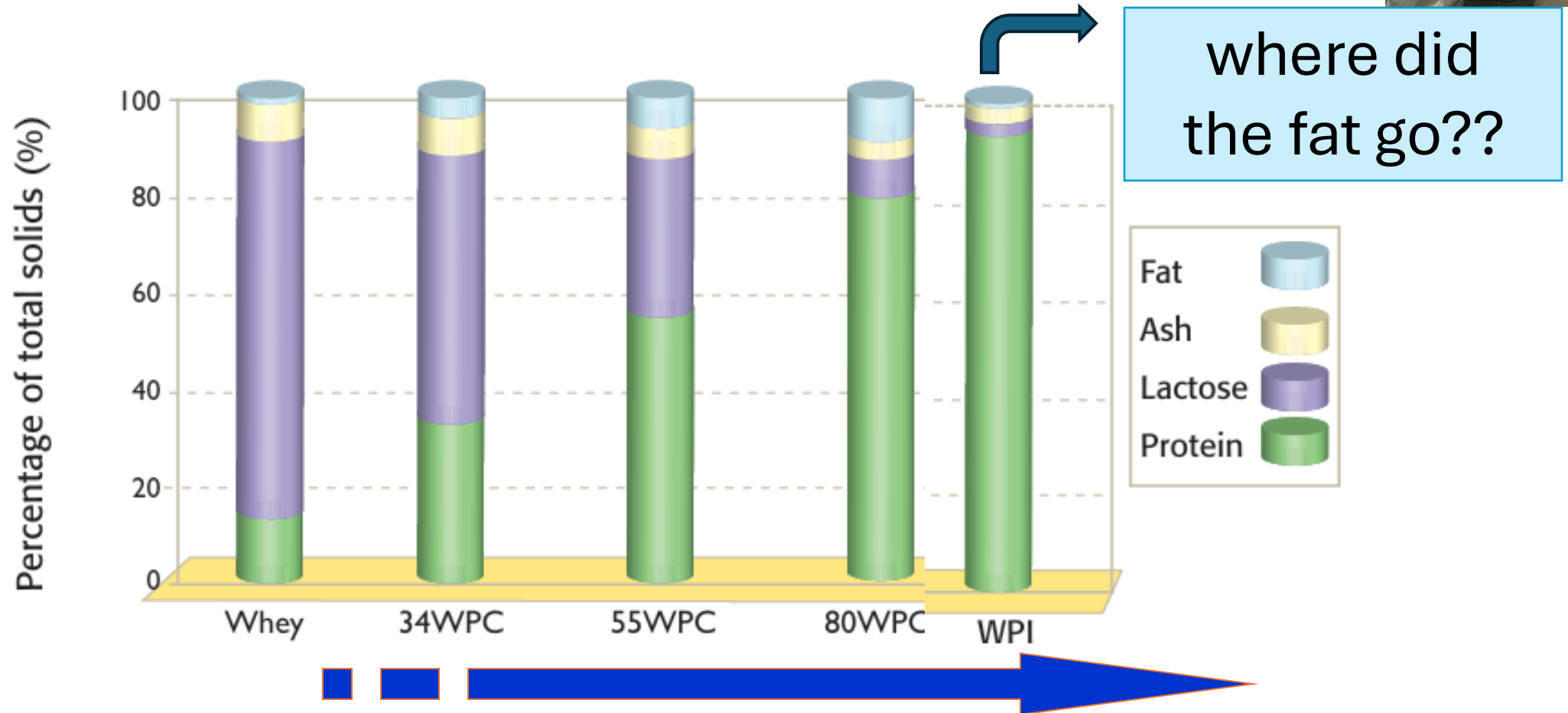
Whey Protein
phospholipid
concentrate



Why do we have new side streams?



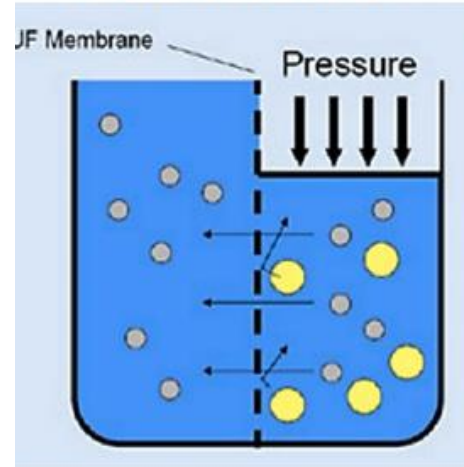
Whey protein concentrate composition



Increasing protein to lactose & fat ratio

whey protein isolates

Microfiltration



Microfiltration of WPC

WPI

Successful
Market \$\$\$

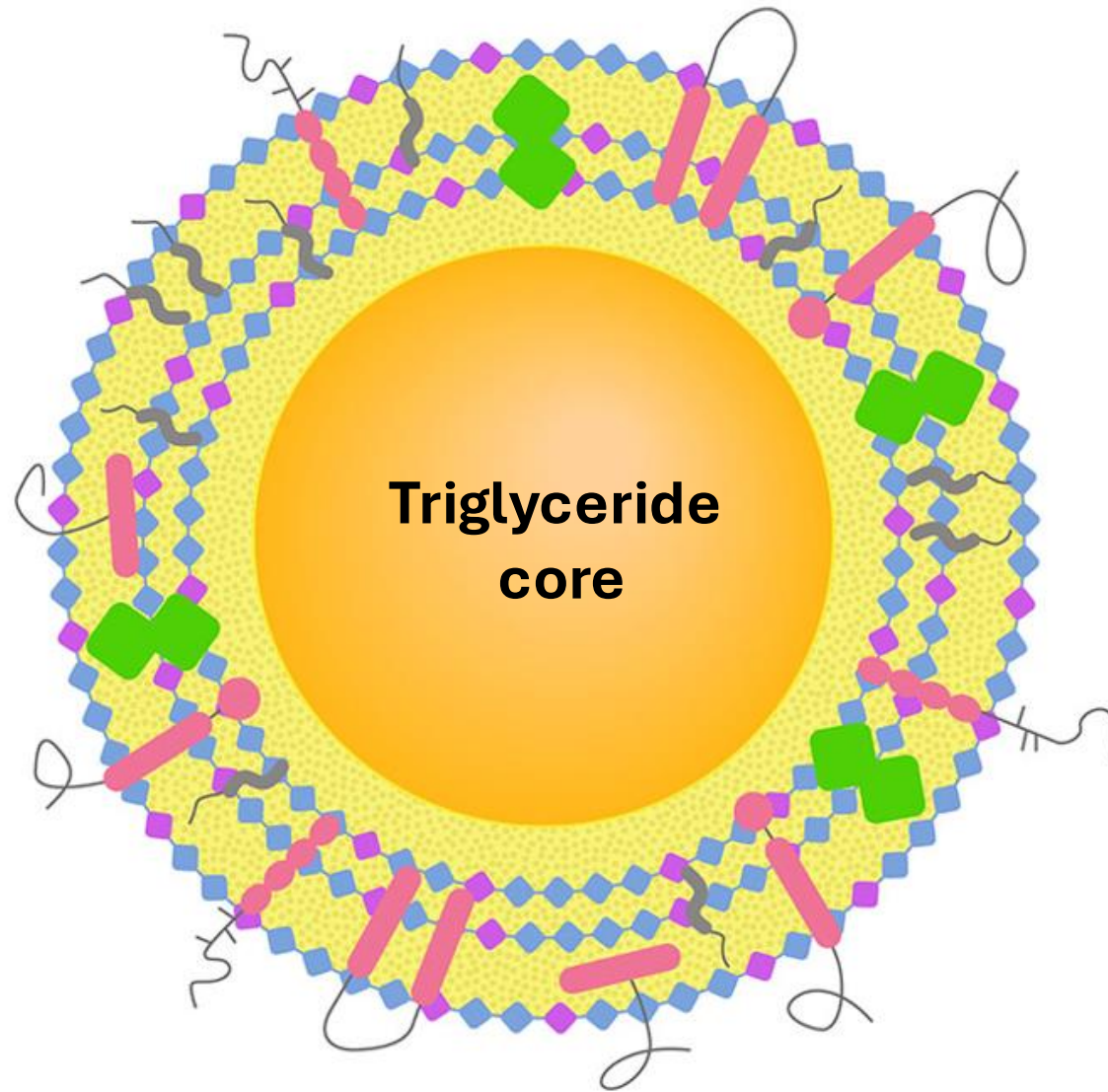
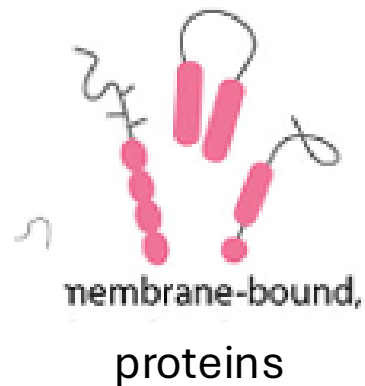


**Whey
Protein
Isolate
(fat free)**

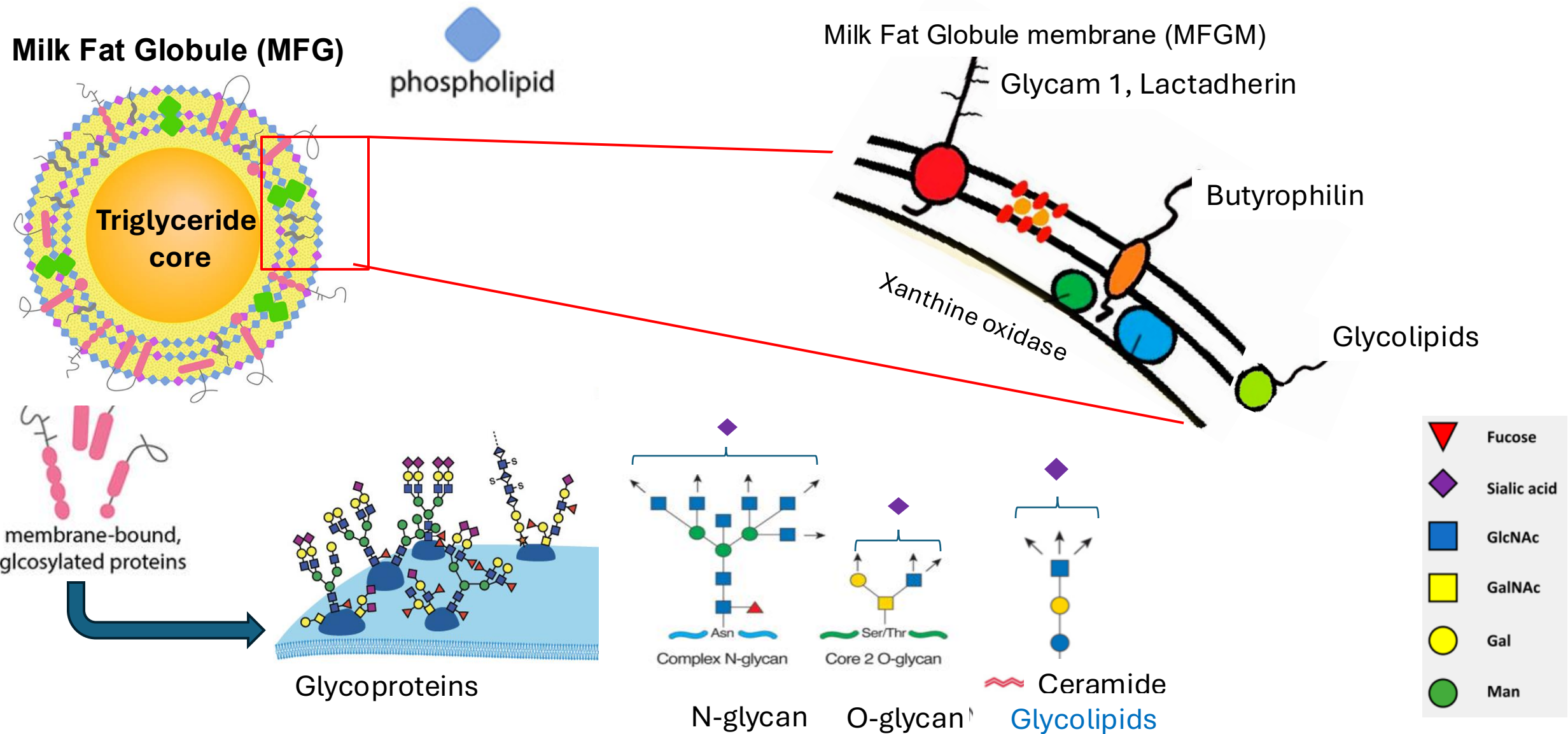


Why is it called **Whey Protein Phospholipid Concentrate**?

Milk Fat Globule (MFG)



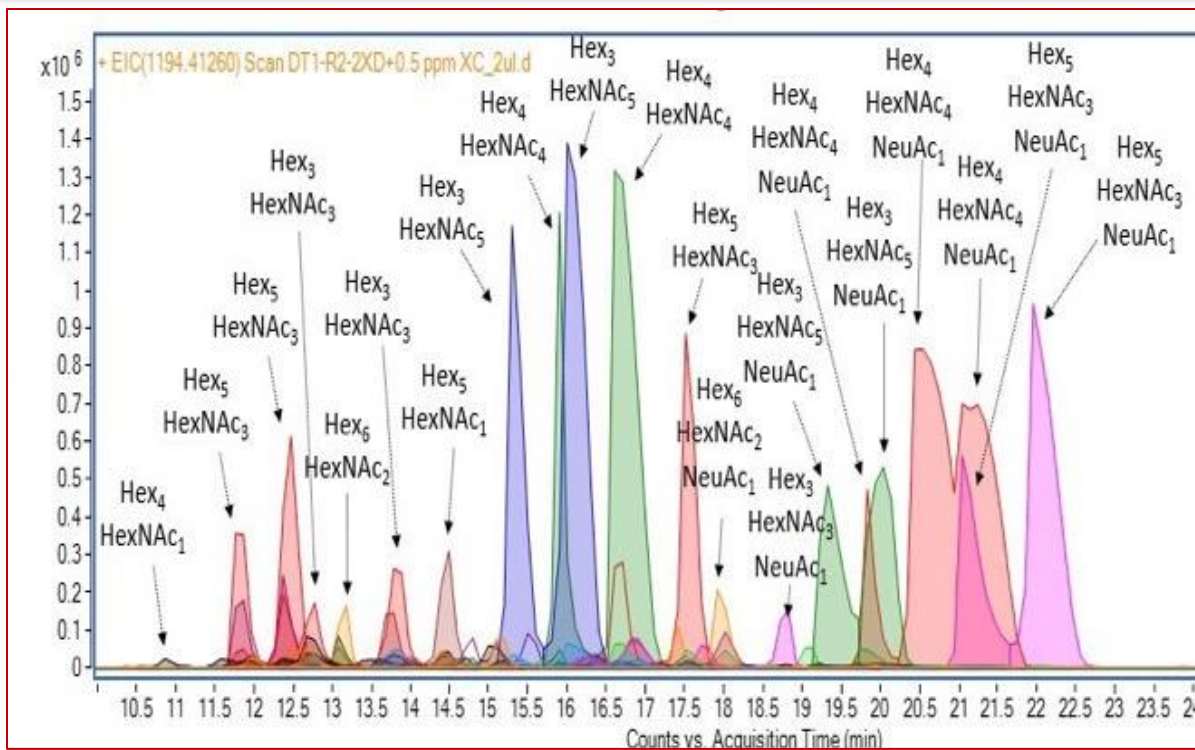
Building blocks for brain embedded into a phospholipid-rich membrane



Glycoproteomic and Lipidomic Characterization of Industrially Produced Whey Protein Phospholipid Concentrate with Emphasis on Antimicrobial Xanthine Oxidase, Oxylipins and Small Milk Fat Globules

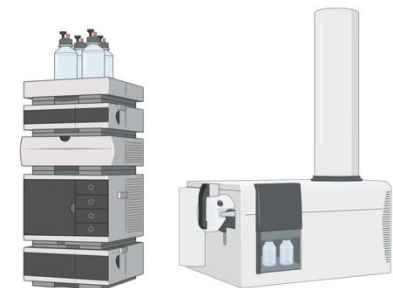
by Gulustan Ozturk ¹ , Nuanyi Liang ¹ , Mrityika Bhattacharya ¹ , Randall C. Robinson ¹ , Shalini Shankar ¹ , Yu-Ping Huang ¹ , Bruna Paviani ¹ , Ameer Y. Taha ¹  and Daniela Barile ^{1,2,*} 

¹ Department of Food Science and Technology, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA



Glycoproteomics analysis revealed 85 N-glycans, many with sialic acid (NeuAc)

>1300 proteins

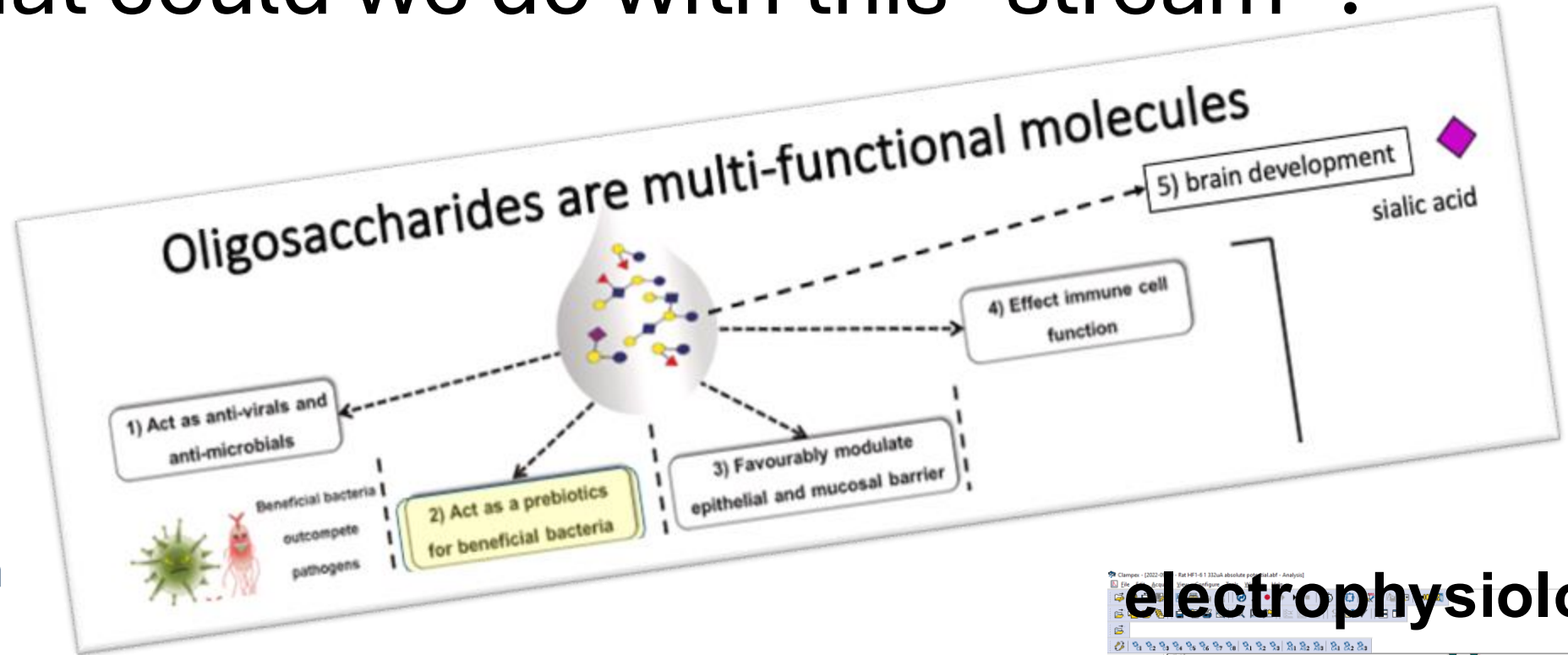


10X increase compared with starting bovine milk!

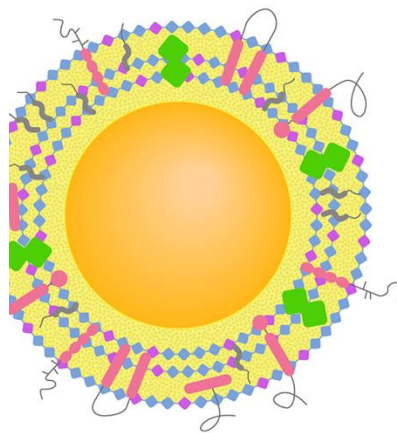
What could we do with this “stream”?



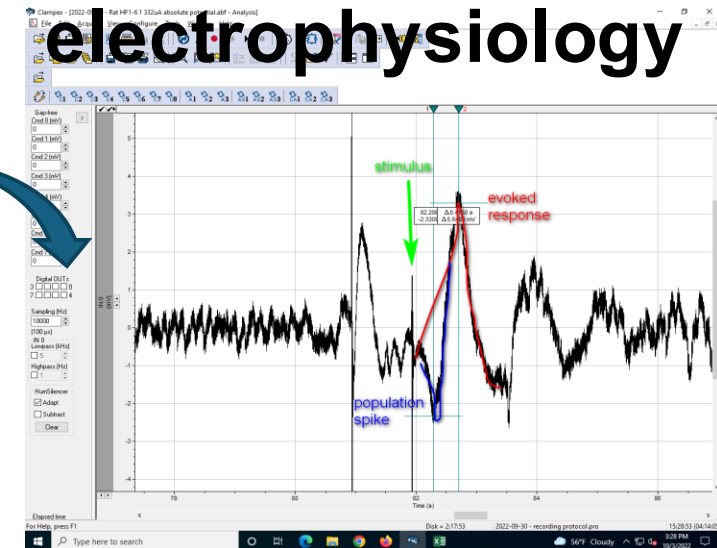
Dr. Ameer Taha
UCD FST



Milk Fat
Globule
(MFG)



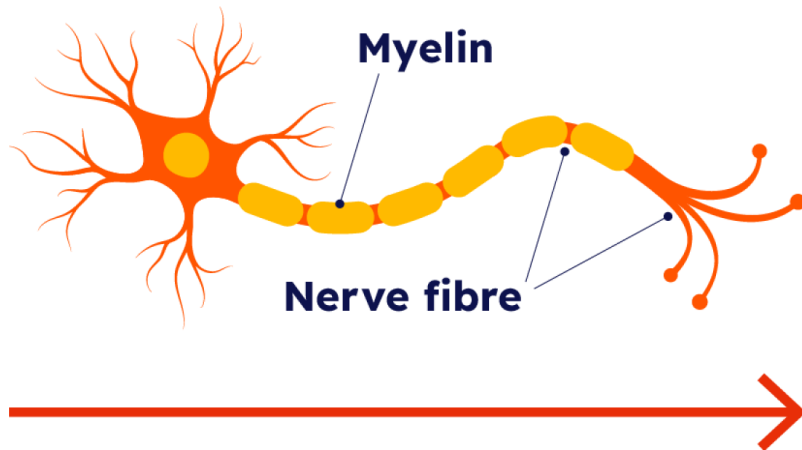
electrophysiology



Hypothesis

- WPPC will improve cognitive function by providing key building blocks important for maintaining brain structure

Note: Myelin turnover & homeostasis is key:



- Too much myelin is bad (lysosomal storage disorders)
- Low myelin (depletion) is also bad (multiple sclerosis)



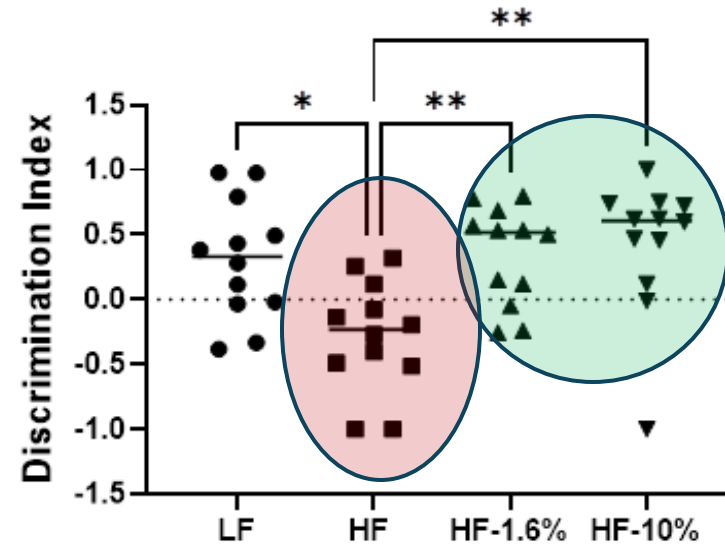
Preventing cognitive deficits caused by high-fat diets

Rats (n=12/group)

Low fat diet [healthy control]
High fat diet [cognitive deficit]
High fat diet + **1.6% WPPC**
High fat diet + **10% WPPC**

Object recognition test

At ~4 months



Higher discrimination index
means better memory

Electrophysiology

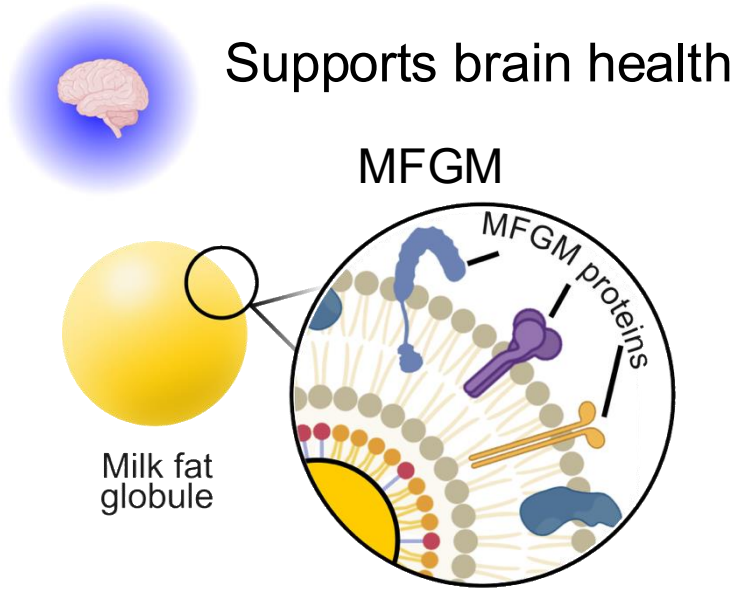


HF diet impaired memory
storage in hippocampus

WPPC improved memory by increasing synaptic connectivity in neurons involved in memory consolidation

P<0.05 by one-way ANOVA followed by Tukey's post-hoc test (n=12 per group)

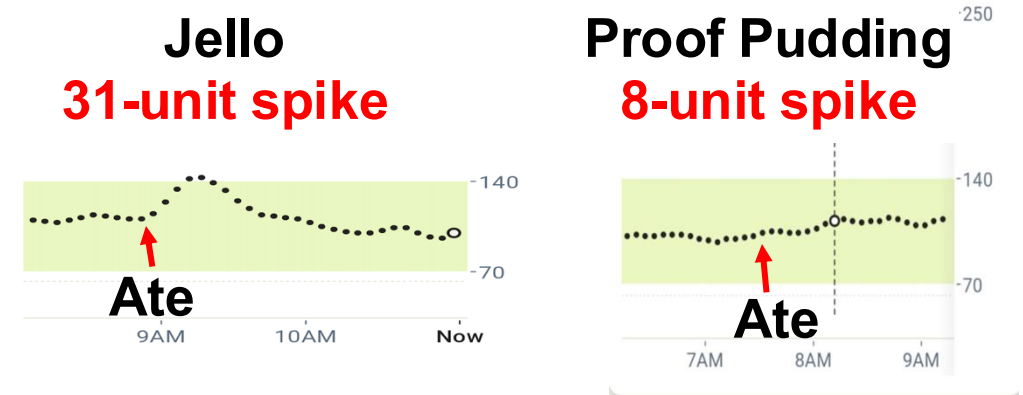
Proof Pudding: A Functional Chocolate Dessert Supporting Brain Health with MFGM



Recent Accomplishments:

- “Proof Pudding” name trademark filed (June 2025)
- Filed provisional patent for formulation (Oct. 2025)
- Real CA Milk 2025 Incubator Program

Proof has a 4-fold lower blood glucose spike than the leading brand



Conclusions

- **Glycomics enables to find valuable bioactives in food streams**
- WPPC, a stream from whey protein isolation, is enriched in phospholipids and sialylated glycans that can yield building blocks for myelin turnover in the brain.
- WPPC prevented cognitive decline induced by a high-fat (HF) diet.
- Now looking into butter streams for these compounds
- Work in progress: rat-studies of **reversing cognitive decline**



California
Dairy
Innovation
Center