PhytoSpherix™: Nature's Nanomaterial

Safe and Natural Nanotechnology for Food Applications

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Mirexus Biotechnologies Inc.

CIFST Fall Technical Session
Mississauga: Oct 28th, 2016

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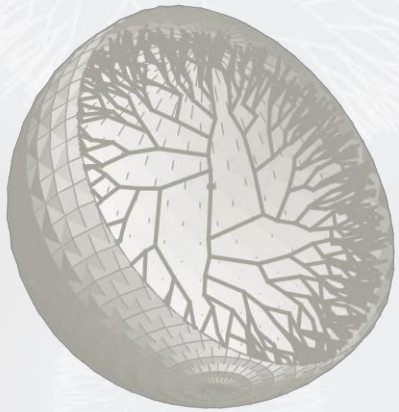
Outline

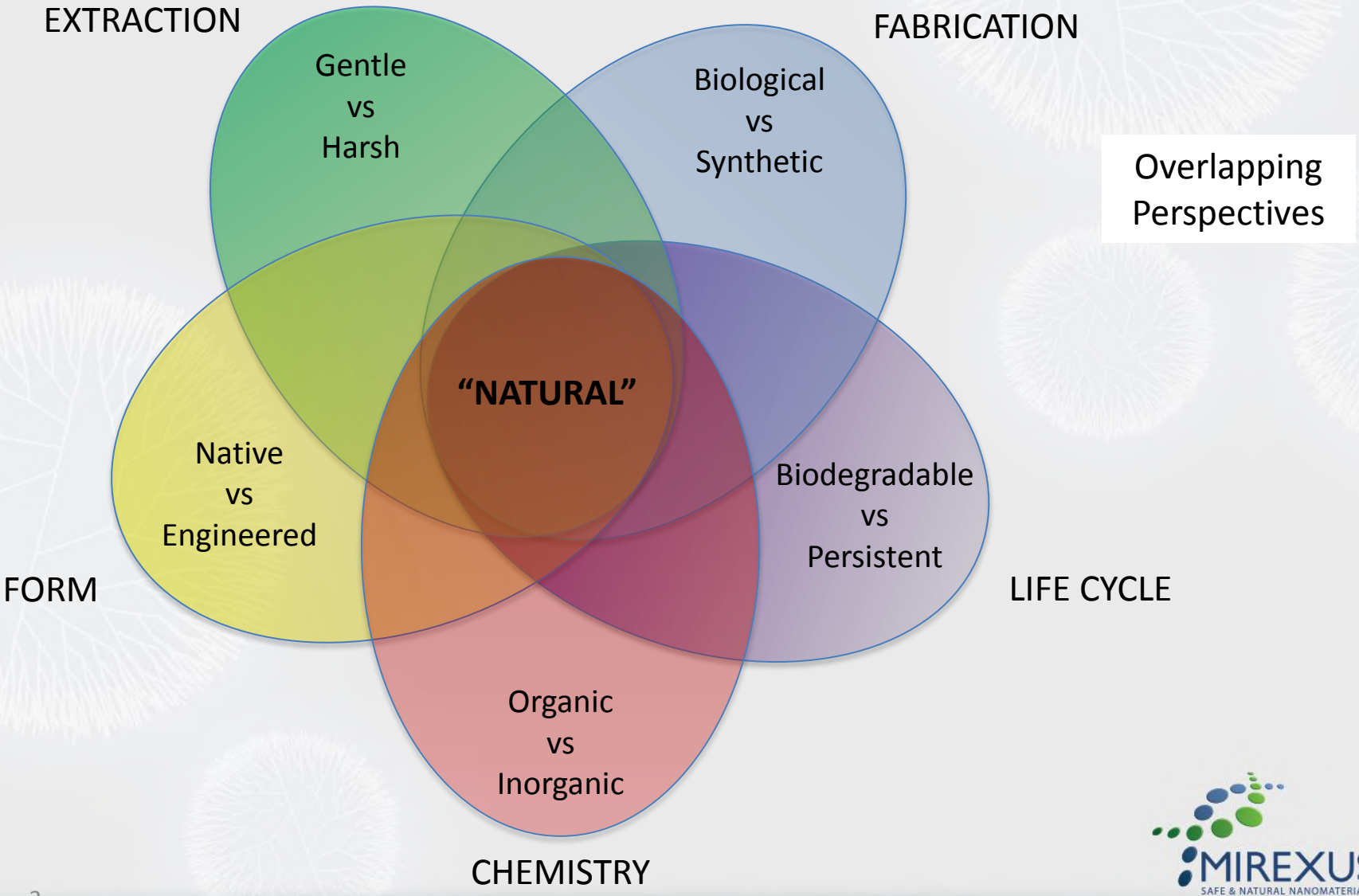
- Introduction
  - What is “Natural”?
  - What is “Nanotechnology”?



- Examples of Natural Nanomaterials

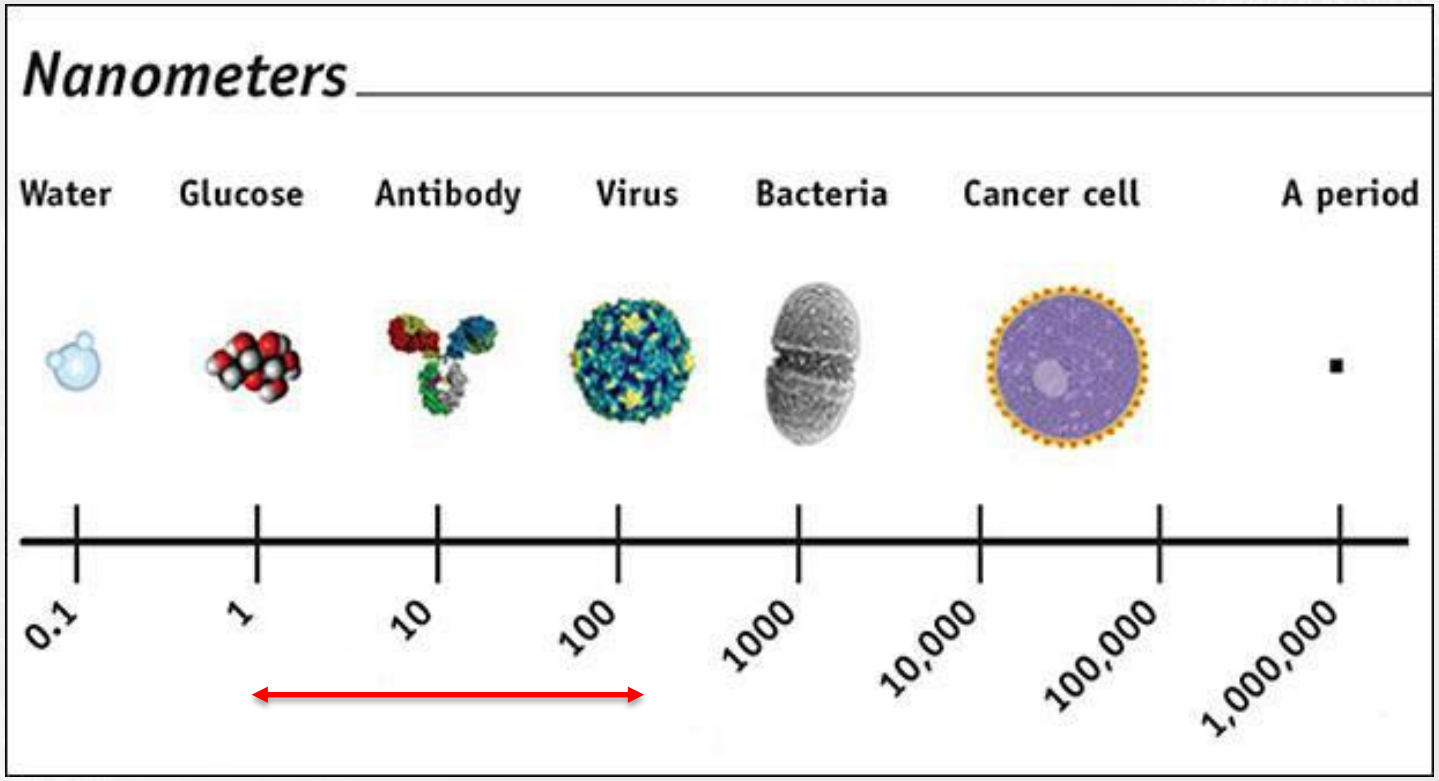
- PhytoSpherix™: Glycogen Nanoparticles
  - Fundamental Properties
  - Applications in Food/Nutraceuticals
  - Applications in Cosmetics and Pharmaceuticals

What is ‘Natural’?



What is ‘Nanotechnology’?

- Simple Answer: Size 1 to 100 nm



Small molecules

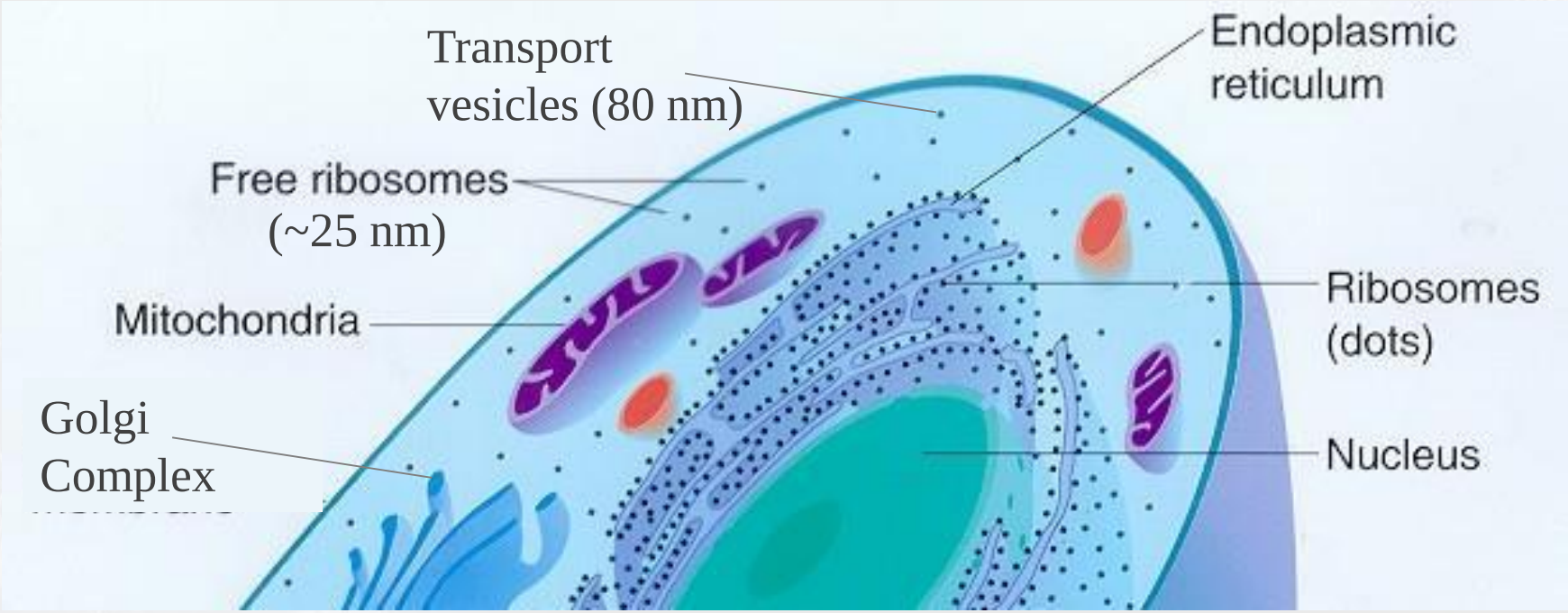
Supermolecular assemblies

Smallest living cells

Eukaryotic cells

What is 'Nanotechnology'?

- Biological Answer: sub-cellular structures
  - Organelles are nanostructures: organize reactions in space
  - Ribosomes, transport vesicles, pleated membranes



- All natural! Just a matter of extraction and purification.

Canada: Regulation of Natural Nanomaterials

Environment Canada: Proposed Approach to Address Nanoscale Forms of Substances on the Domestic Substances List

*"Proposed criteria for substances to be **excluded**, unless they are intentionally manufactured to exhibit one or more nanoscale properties/phenomena, include, but are not limited to:*

- ***Polymers**;*
- *Organic or organo-metallic pigments and dyes;*
- ***Naturally occurring** or incidentally produced nanomaterials;*
- *Deoxyribonucleic acid, ribonucleic acid, proteins, peptides, liposomes, antibodies, viruses or a virus-like particles, organelles or other **biological materials**."*

PHYTOSPHERIX meets these criteria and we expect it will be exempt from regulation as a nanomaterial – as would other natural nanomaterials

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Commercialization: Regulatory Compliance

PhytoSpherix™ is not classified as a nanomaterial by cosmetic regulatory and certification bodies (Health Canada, EU, COSMOS, ACO etc.) because:

- PHX is **water soluble** (individual molecules remain in solution indefinitely)
- PHX is **not biopersistant** – the material is quickly and easily degraded to simple sugars in the environment and in the body
- PHX is **not artificially engineered** – it is simply extracted from corn in the same state as it is found in nature

PHX is a botanical extract from non-GMO sweet corn.

From a regulatory perspective: it's "non-nano nano"

Health Canada approved PhytoSpherix™ as a food ingredient in ***36 days***

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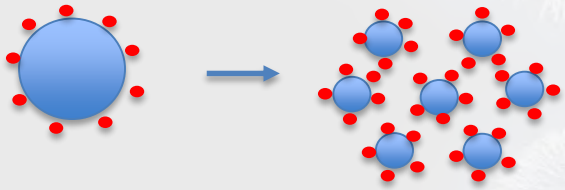
Regulation of Natural Nanomaterials

The Australian View:

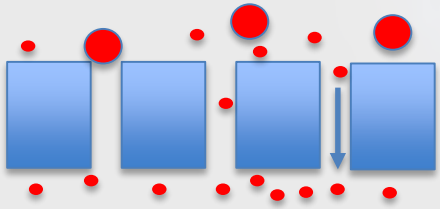
Nanotechnology includes the intentional manufacture of material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale. **This does not include the presence in certified organic products of naturally occurring nanoparticles**, for example from nanoparticles in volcanic soils, or **incidentally produced (nonmanufactured) nanoparticles**, for example, occurring in **flour** as a byproduct of the traditional milling process.

Functional Benefits of Nanotechnology

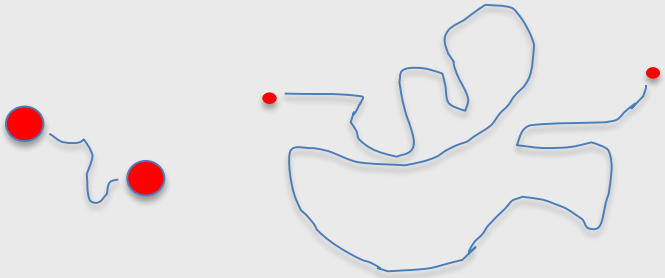
- What happens when you make objects very small?
  - More SURFACE AREA
    - Surface-catalyzed reactions
    - Surface-bound ligands



  - Higher PENETRATION
    - Cellular uptake
    - Inter-cellular spaces
    - Smoothing and filling

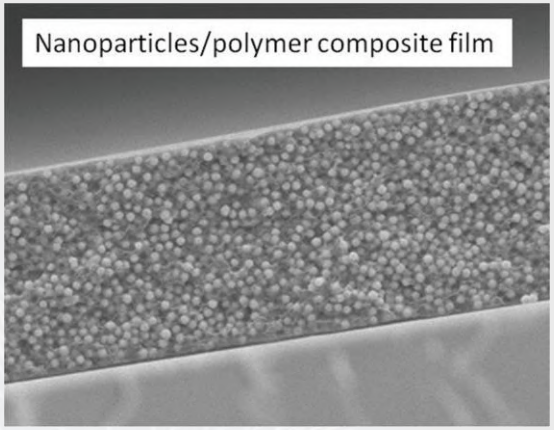


  - Higher MOBILITY
    - Faster diffusion
    - Longer distances



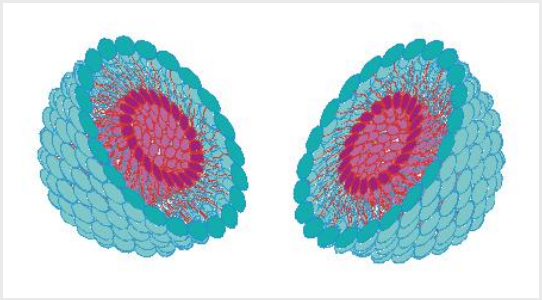
Examples of Synthetic Nanotechnology

Nanoparticle Additives



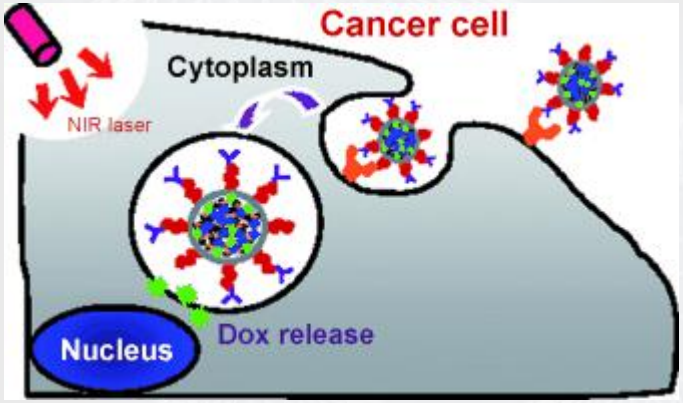
https://unit.aist.go.jp/amri/group/e-cera/en/contents.html

Nano-Liposomes



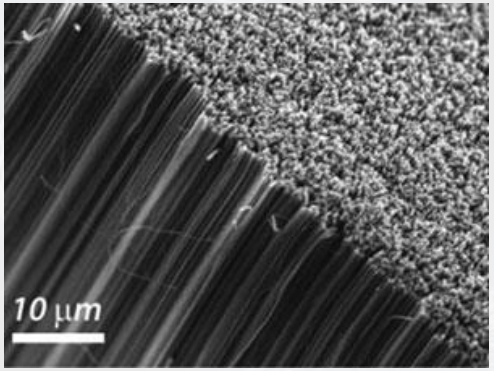
http://www.elsomresearch.com/learning/technology/nanosomes.htm

Nanoparticle Vehicles



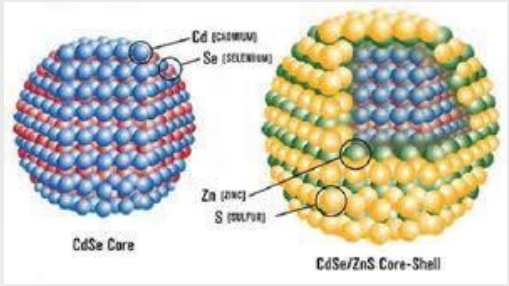
A Drug-Delivery Vehicle Combining the Targeting and Thermal Ablation of HER2+ Breast-Cancer Cells with Triggered Drug Release

Nanotubes



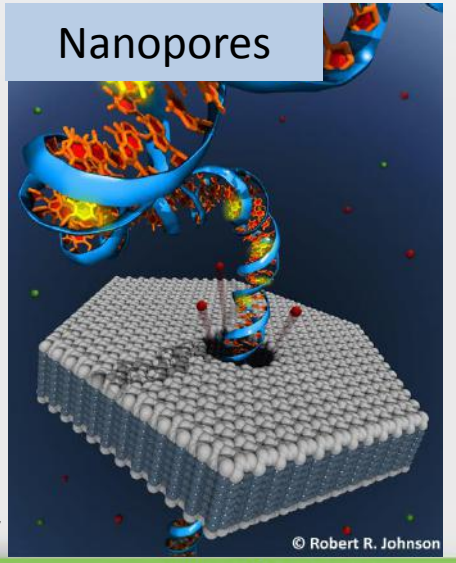
http://www.rsc.org/chemistryworld/2012/06/challenging-consensus-nanotube-electrochemistry

Quantum Dots



http://www.infobarrel.com/Quantum_Dot_Fluorescence

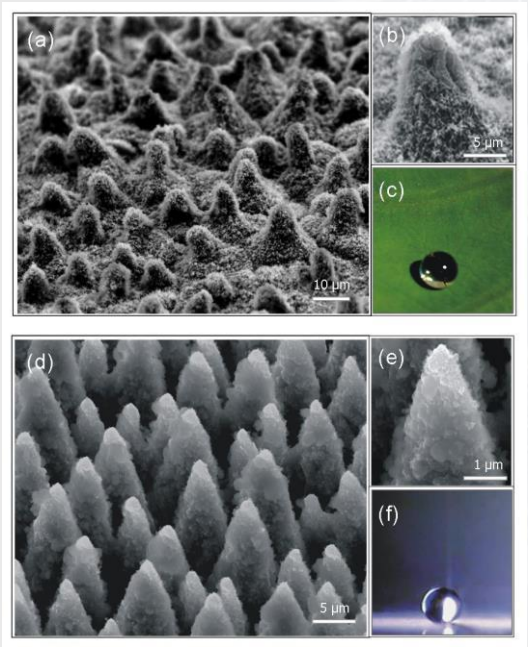
Nanopores



http://www.physics.upenn.edu/~robertjo/gallery/

Examples of Biomimetic Nanotechnology

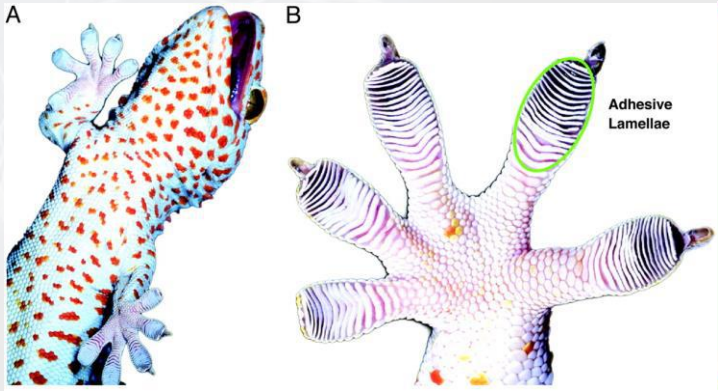
Surface Coatings and Patterns

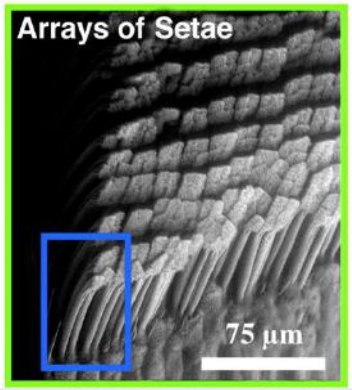


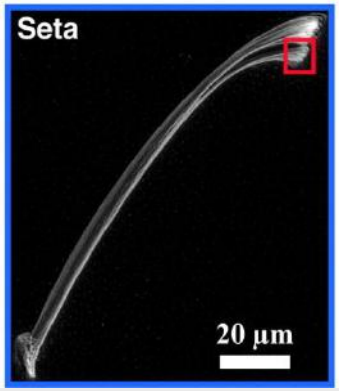
Lotus Leaf:
water repellent and self-cleaning super-hydrophobic surface

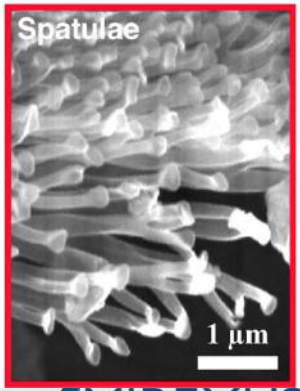
Gecko Feet:
hierarchically structured super-adhesive surface

http://www.photonics4life.eu/Consortium/P4L-DB/All-items/Ultrafast-laser-based-fabrication-of-biomimetic-micro-nano-structured-surfaces









http://galleryhip.com/gecko-toes.html

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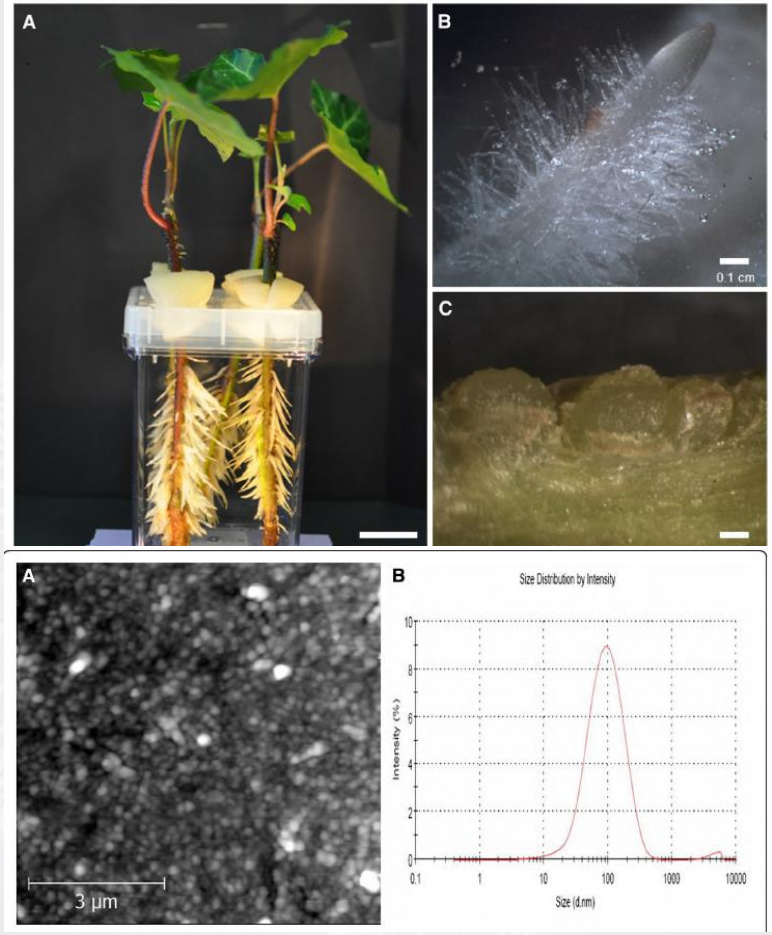
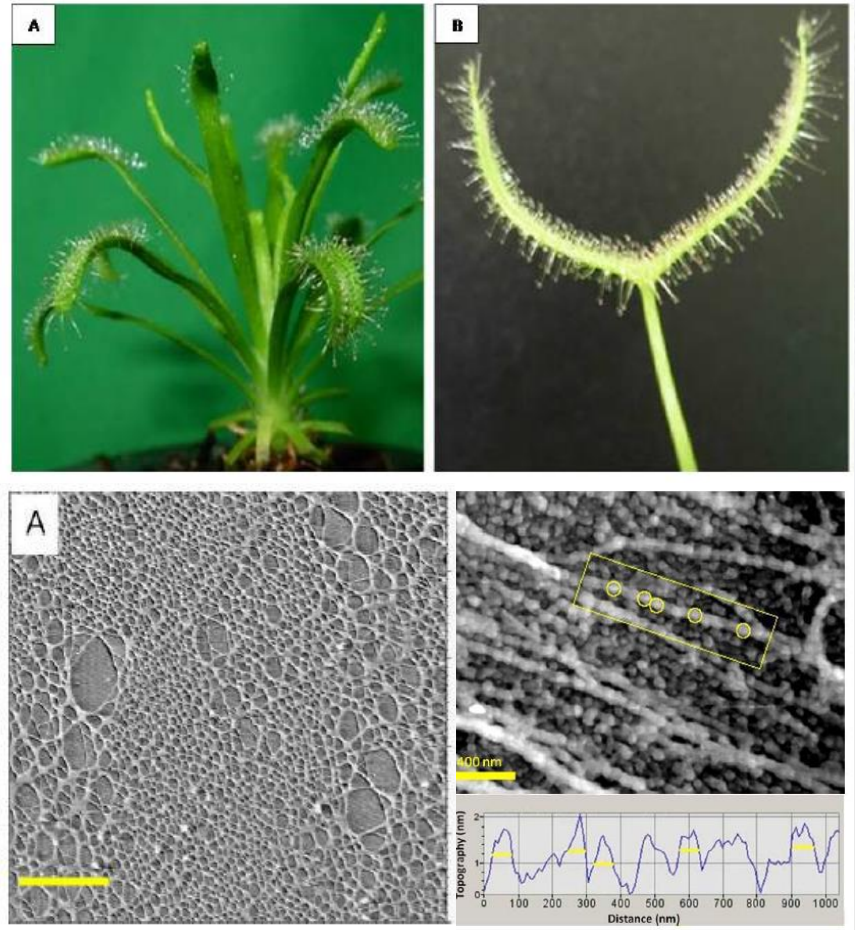
Natural Adhesive Nanoparticles

Ivy



Sundew



Dr. Mingjun Zhang, Ohio State University
Nano-Biosystems and Nano-Bio-Inspired Device Lab

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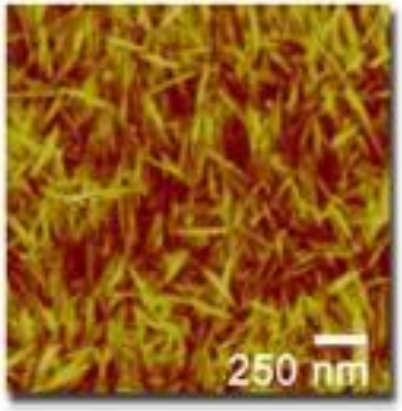
Nano-Cellulose

- Biocompatible, non-toxic & biodegradable
  (Kovacs et al. *Nanotoxicology* **2010**, 4, 1)
  - First approved safe nanomaterial (Domestic substances list, Environment Canada 2012)
- Chemical properties
- Optical and barrier properties

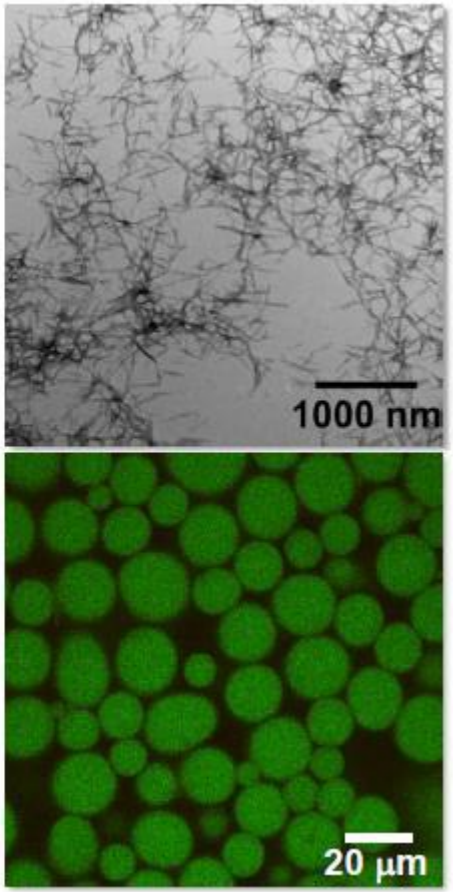


- Mechanical properties (high degree of crystallinity)
- Natural, renewable, sustainable
- Easily produced in industrial-scale quantities (green)
  - Industrial production (kg to ton per day quantities)

Dr. Emily Cranston: McMaster University
Sustainable Composites: Nano-Bio-Lab

Nano-Cellulose

- Large **aspect ratio** and high surface-area-to-volume ratio (surface area 250 m^2/g)
  - ✓ A little goes a long way!
- Ideal **reinforcing** component in nanocomposites
  - ✓ Strong but flexible (specific modulus > steel & Kevlar)
- Ideal **stabilizing** agent in foams, gels, and emulsions
  - ✓ Intermediate hydrophilicity



Dr. Emily Cranston: McMaster University
Sustainable Composites: Nano-Bio-Lab

Safe and Natural Nanotechnology

PhytoSpherix™ from Mirexus

The world's only Monodisperse, Natural Nano-Glycogen

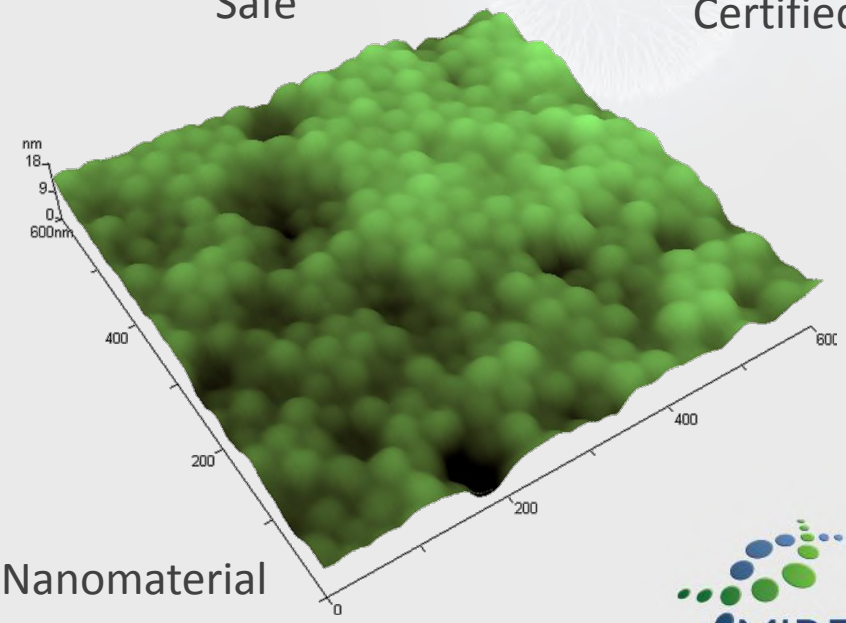
Mirexus has solved the safety and regulatory problems posed by nanotechnology through developing a new nanomaterial that is produced from non-GMO corn and is completely safe.

Safe

Certified

100% Natural

Biodegradable



Nanomaterial

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Nano-Glycogen: PhytoSpherix™ from Mirexus

Safe & Natural Botanical Extract from Corn

- New Nanotechnology:
  - *Natural origin*
  - *'Bioidentical'*
  - *Monodisperse*
  - *Versatile*
  - *Scalable*
- Patent Protected
- Applications safe for human use
  - *Personal Care*
  - *Food and Nutraceutical*
  - *Pharmaceutical*

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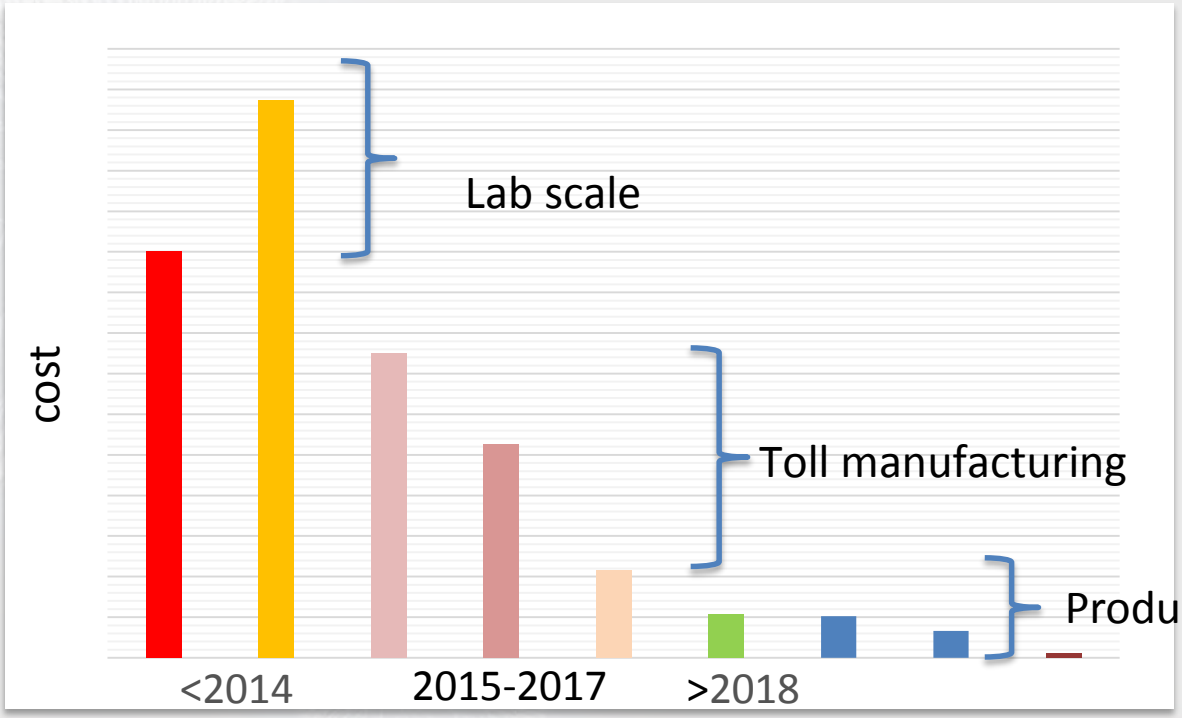
Manufacturing And Scalability

- Level of purification determines domain of application

Food Grade	$
Cosmetics Grade	$$
Pharmaceutical Grade	
Analytic Grade	$$$



Mirexus is currently engineering a pilot plant and process design facility in Guelph, which will be complete by 2018. It will have the capacity to produce 4 Tonnes per year

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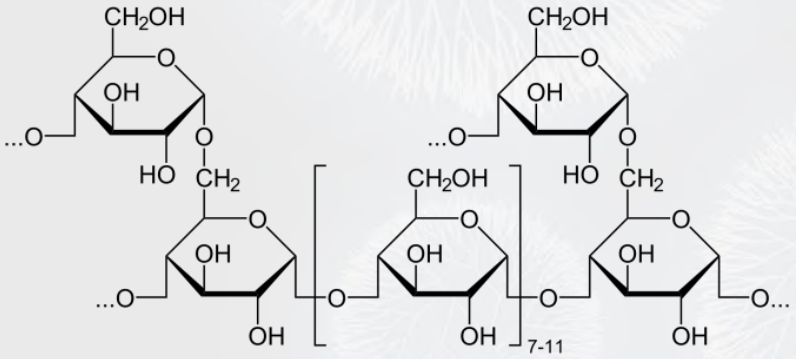
Nano-Glycogen: PhytoSpherix™ from Mirexus

- **Glycogen** is a natural material found in most living organisms
  - One of nature's ways to store energy
  - A natural polysaccharide (sugar)

  Glycogen is commonly extracted from
  - Oysters, Mussels & Clams
  - Liver
  - Bacteria



Branching Dendrimer:

10 glucose units per chain

(amylopectin: 20)

- **Animal glycogen:**
  - Difficult to extract and purify (animal proteins)
  - Polydisperse

- **Plant glycogen: native form is a monodisperse particulate material**
  - Can be isolated with no chemicals
  - Low proteins & lipids, no endotoxins
  - No glycogen debris

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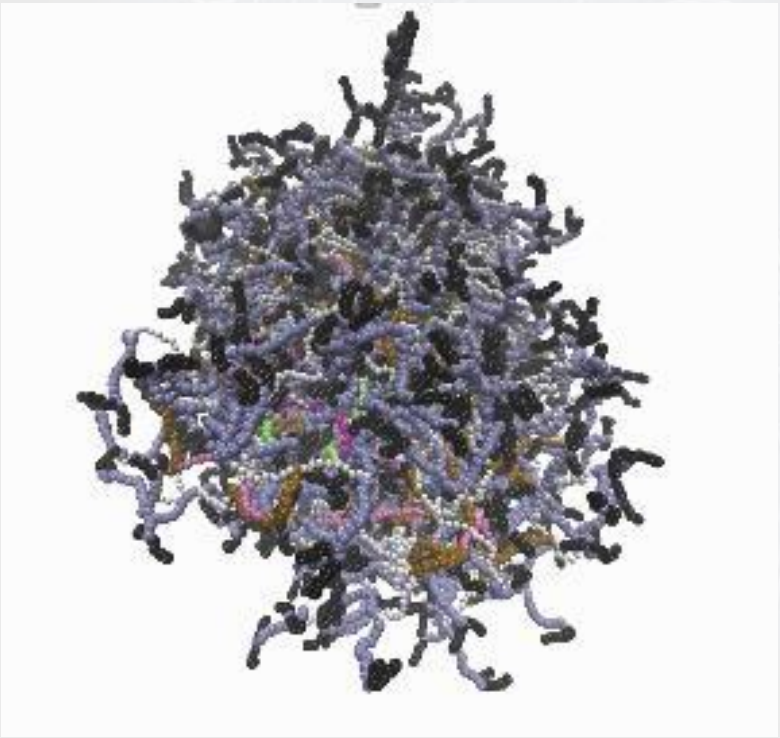
Fundamental Properties

- Glycogen nanoparticles
- Chemically modifiable
- Safe, non-toxic, and edible
- Monodisperse: uniform size
- Biodegradable and Digestible
- Highly water soluble:
  - up to 25% loading before viscosity increase
- Porous structure
  - Can be filled with small molecules
- Soft and Malleable
  - More like a liposome than a nanoparticle



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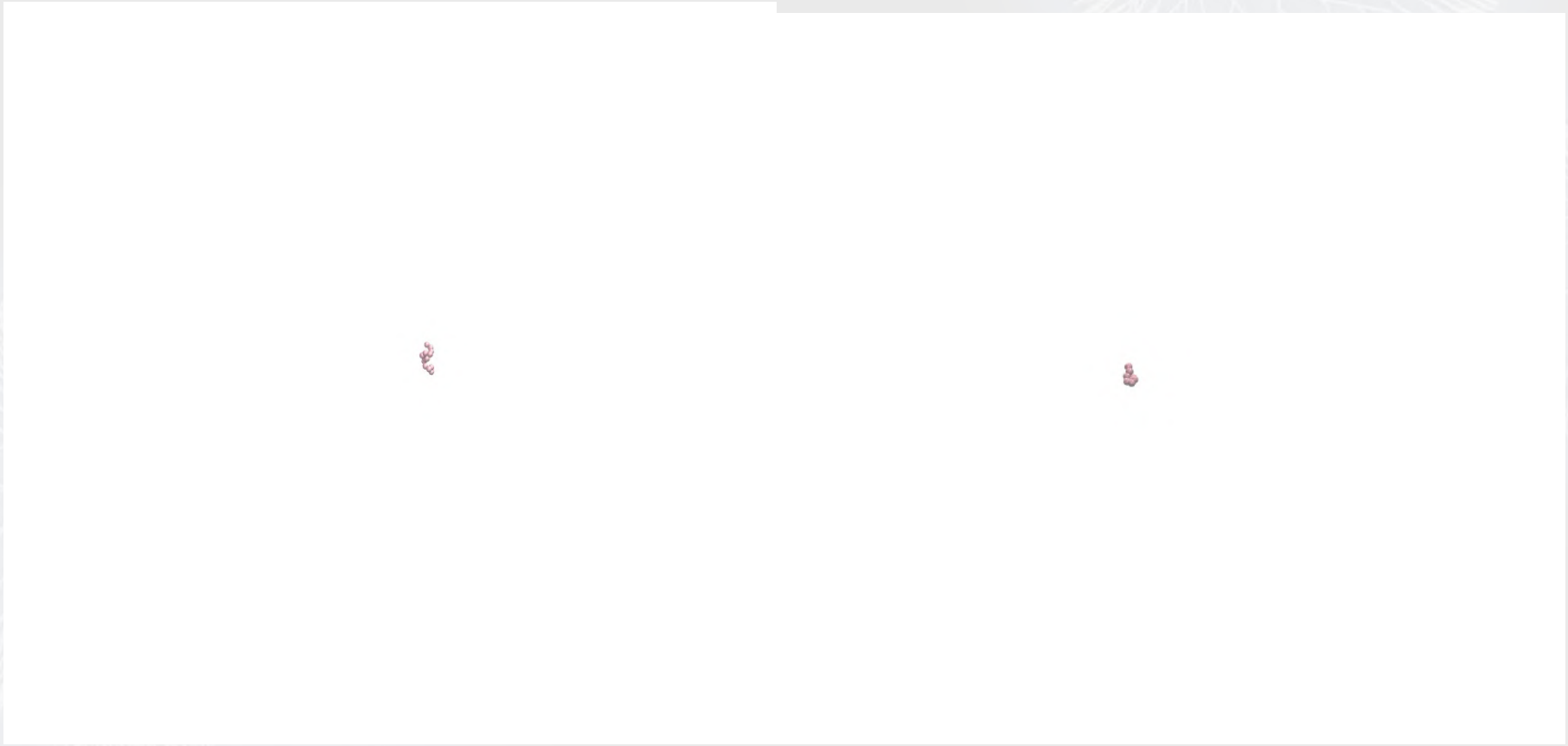
Fundamental Properties: Simulations

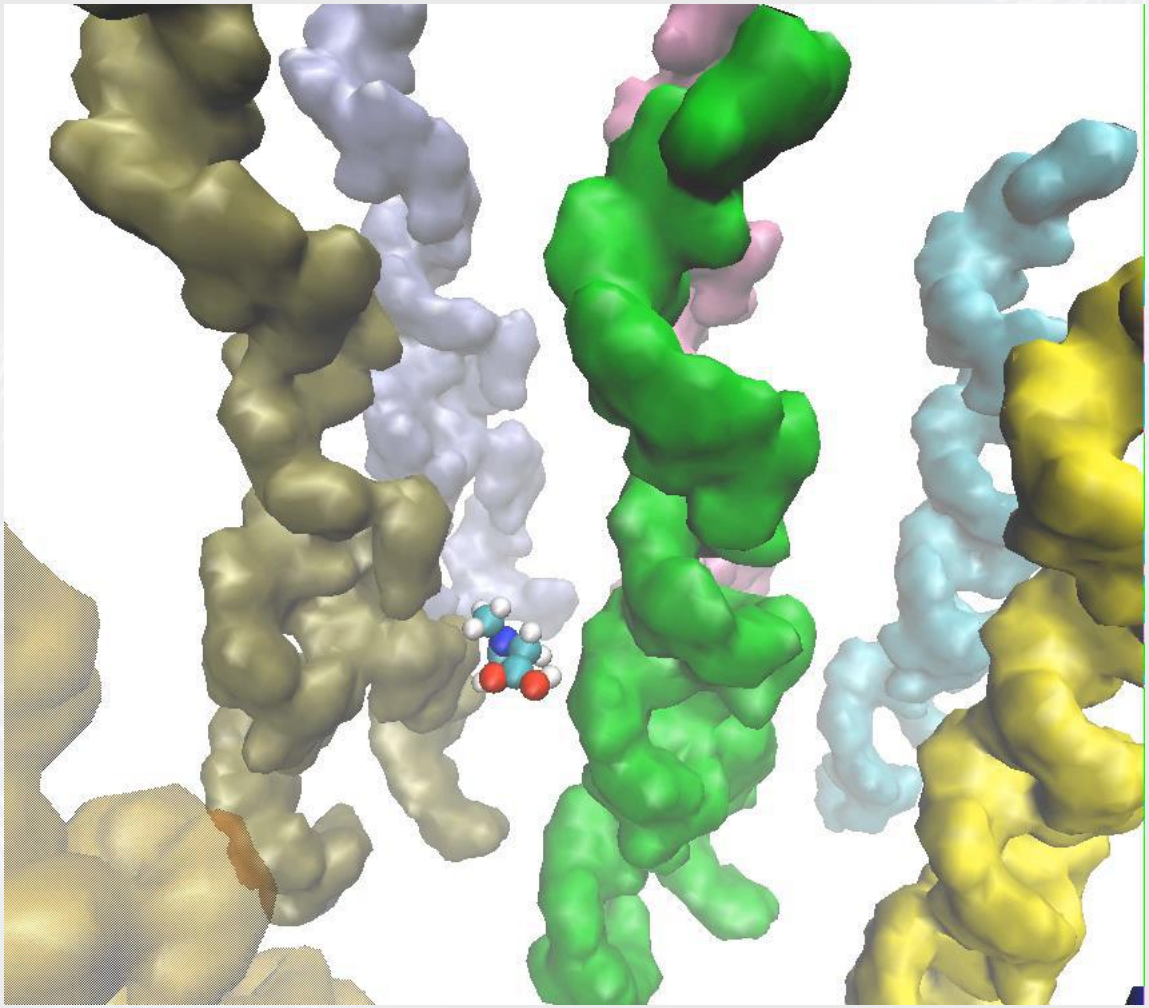
Coarse Grained Growth Models: 1 glucose per bead



*UOIT: Professor **Henk DeHaan**, OCE VIP1 + NSERC Engage*

Fundamental Properties: Simulations

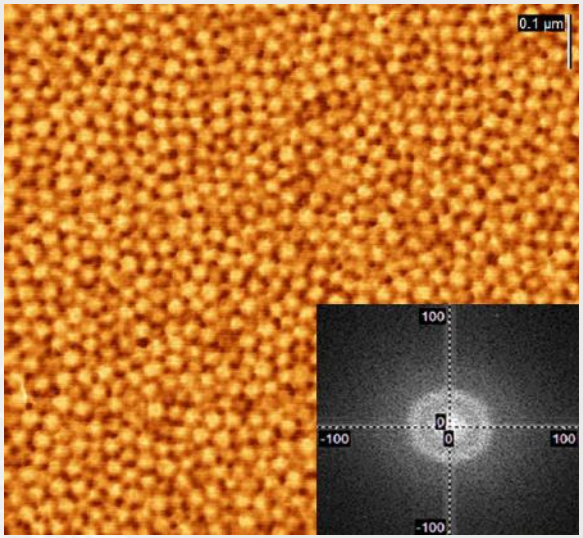
Atomistic Chain and Ligand Modeling



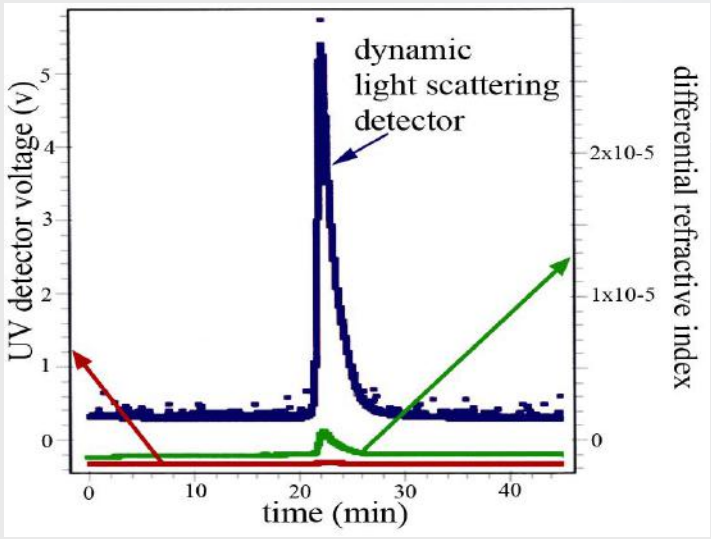
*UOIT: Professor **Henk DeHaan**, OCE VIP1 + NSERC Engage*

Fundamental Properties: Monodispersity

- Biology does the hard work of nano-fabrication
- The particles are all the same size and shape
- **Polydispersity ratio as small as 1.005.**



AFM and Fourier Transform



Size Exclusion Chromatography

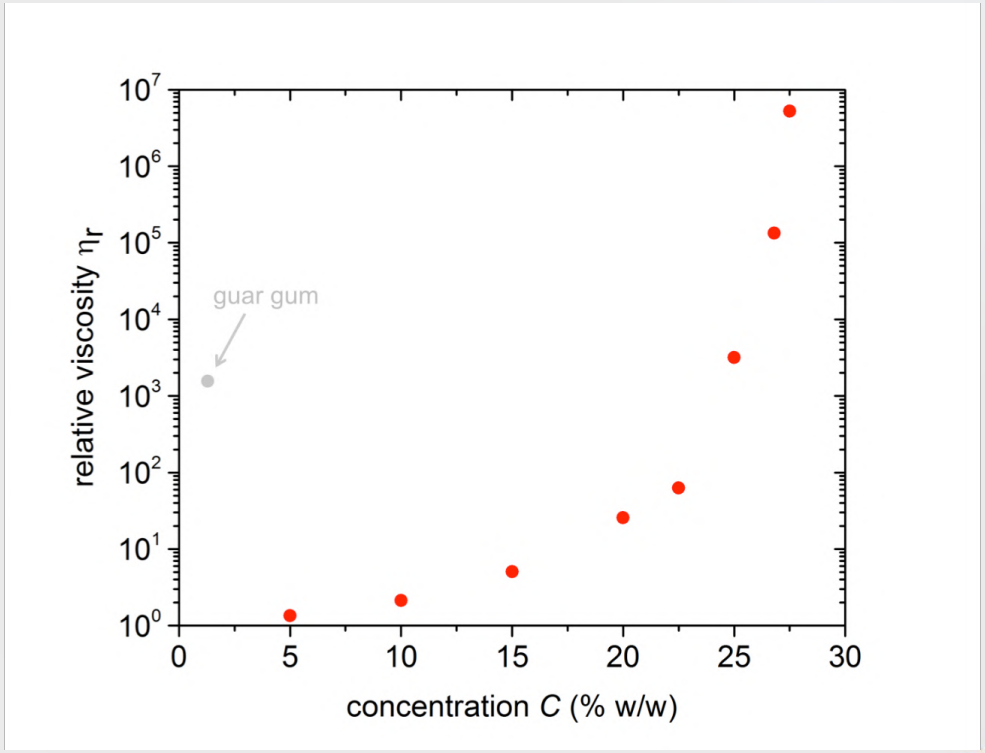
Monodispersity is essential in biomedical applications
… yet normally difficult and expensive to achieve

*University of Guelph: Professor **John Dutcher***

Fundamental Properties: Rheology

Rheology measurements show little change in viscosity until ~25% loading

PHX acts like **hard** spheres in aqueous dispersions



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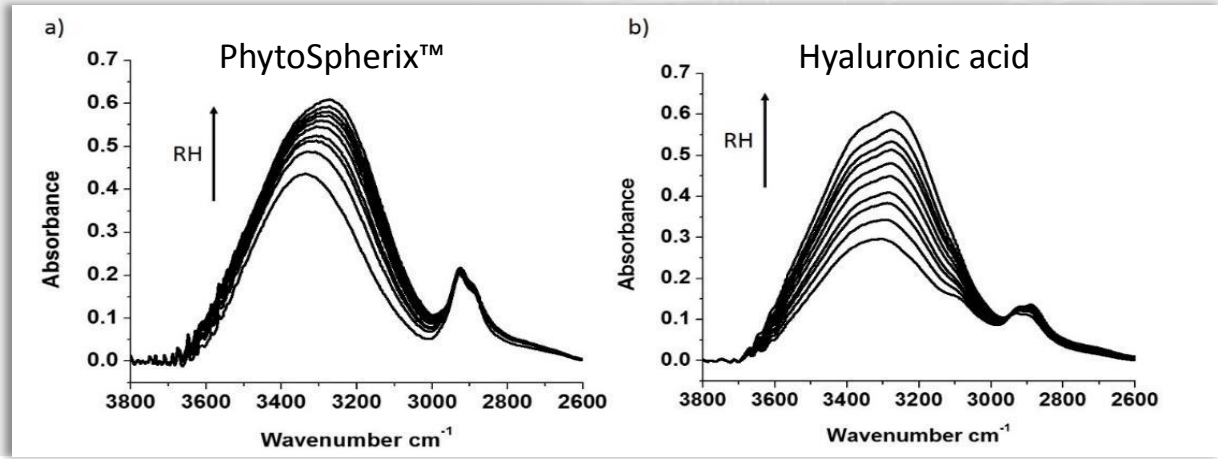
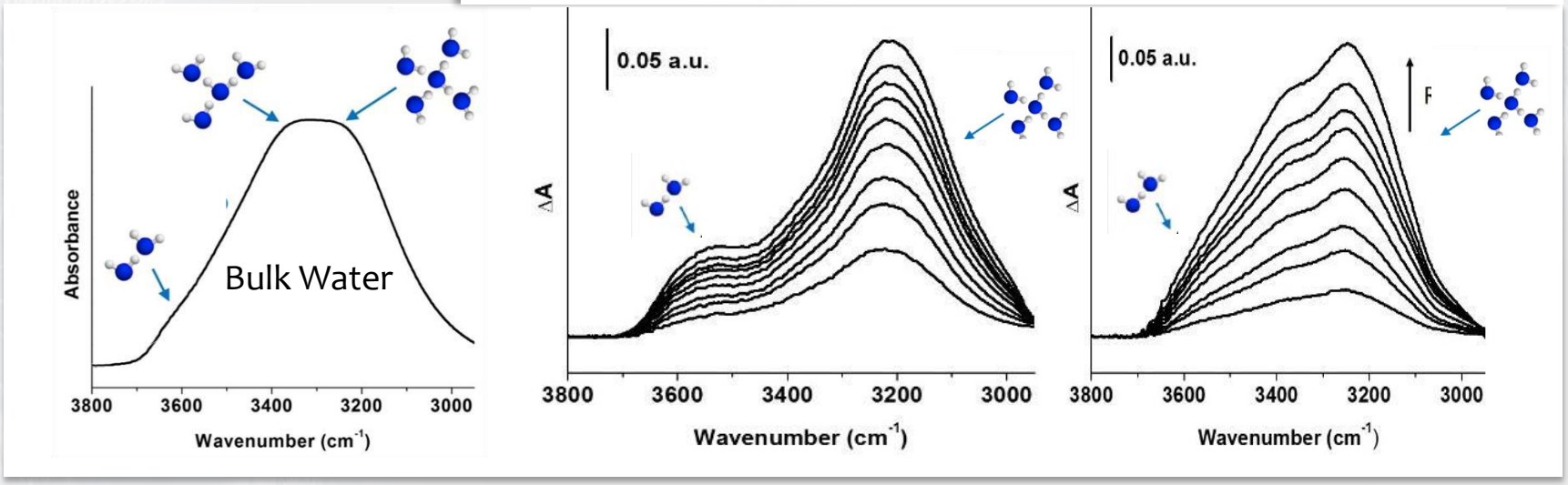
Fundamental Properties: Hydration

Infrared spectroscopy shows that PHX has more strongly bound water than HA





Water in PHX is more **ordered** and more **strongly bonded** than in HA

*University of Guelph: Professor **John Dutcher** -* Grossutti & Dutcher, Biomacromolecules (2016)

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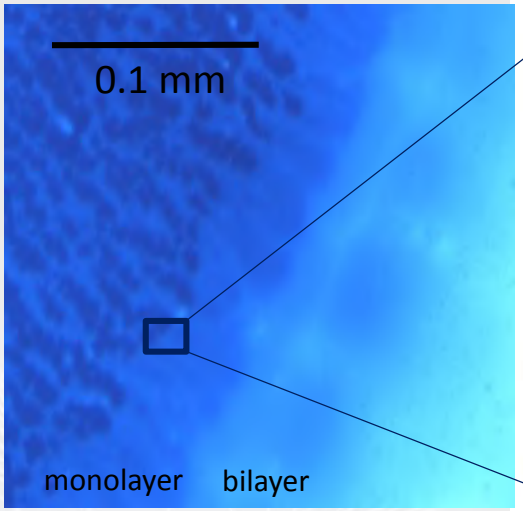
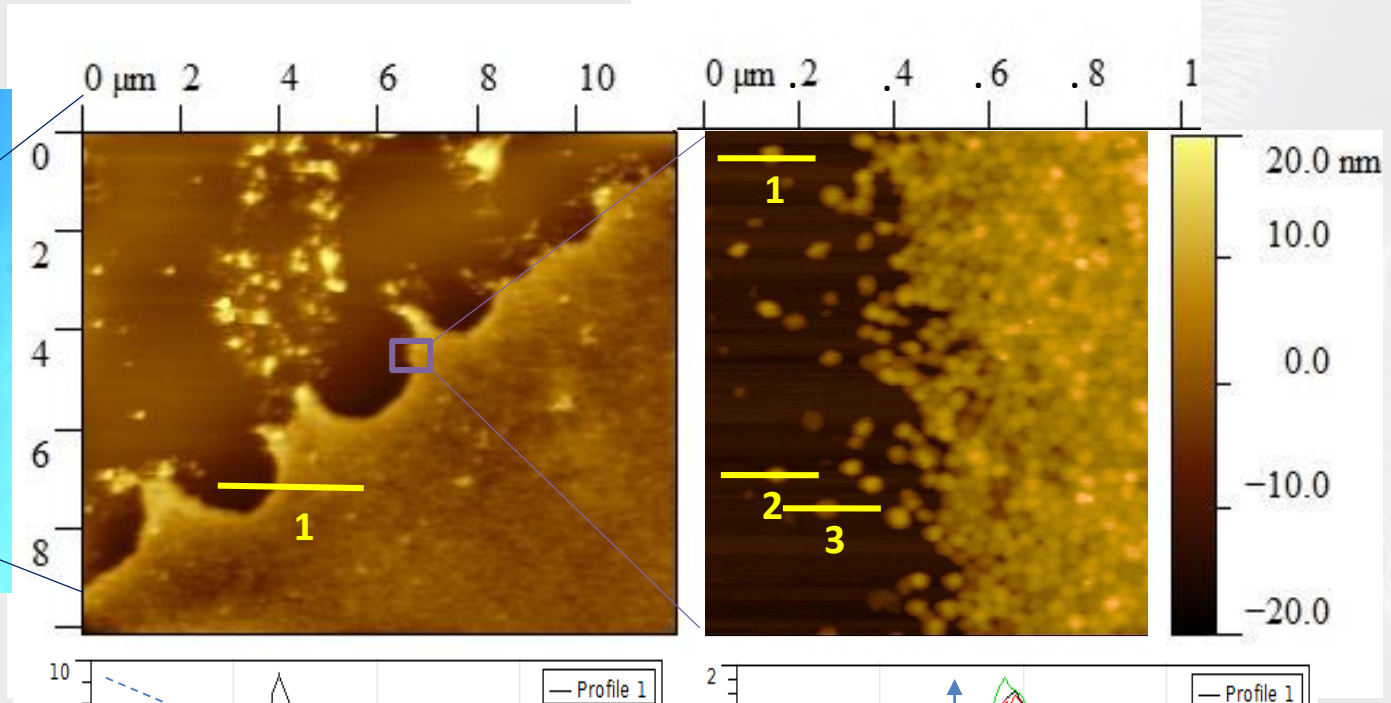
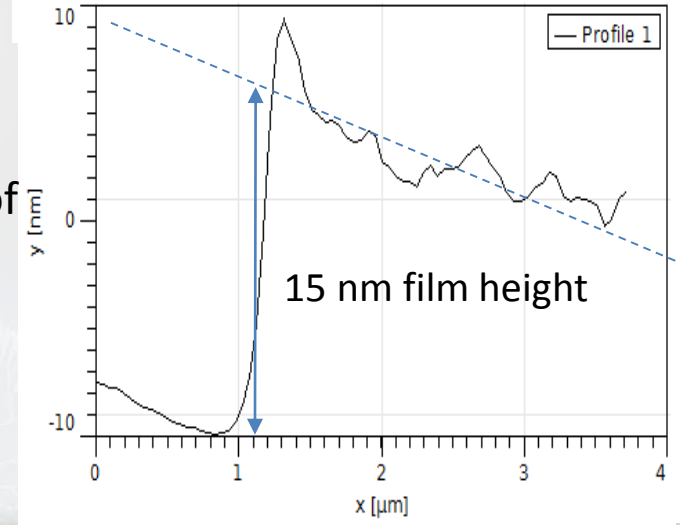
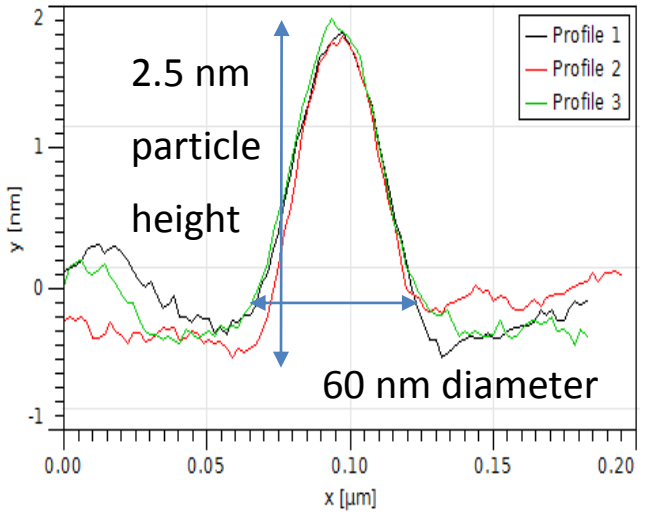
Fundamental Properties: Film Formation



Reflected white light microscopy, 100 x





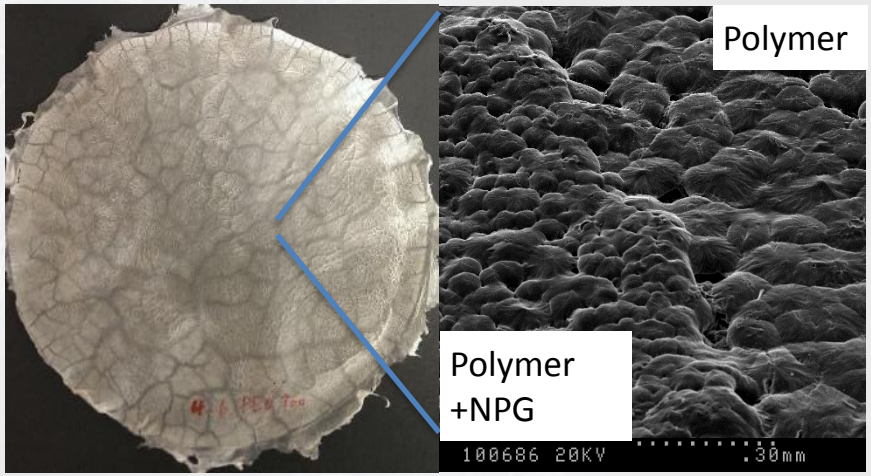


- PhytoSpherix™ nanoparticles self-assemble into layers of close-packed spheres
- Films are edible, hold moisture, and can be nanometers thin

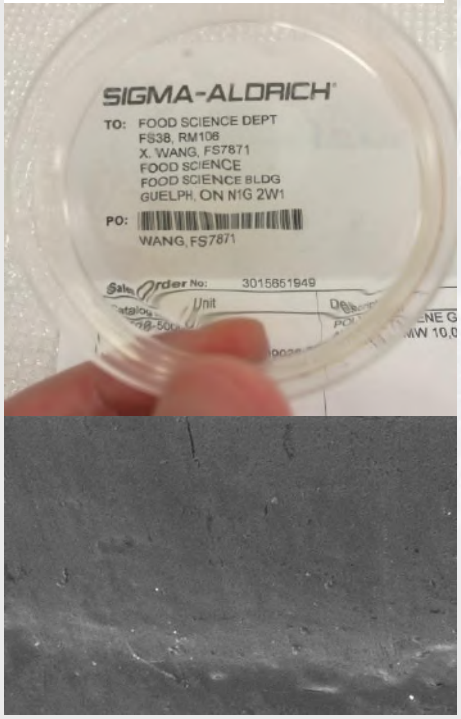
Fundamental Properties: Film Formation

Flexible, Free-standing film of pure PHX

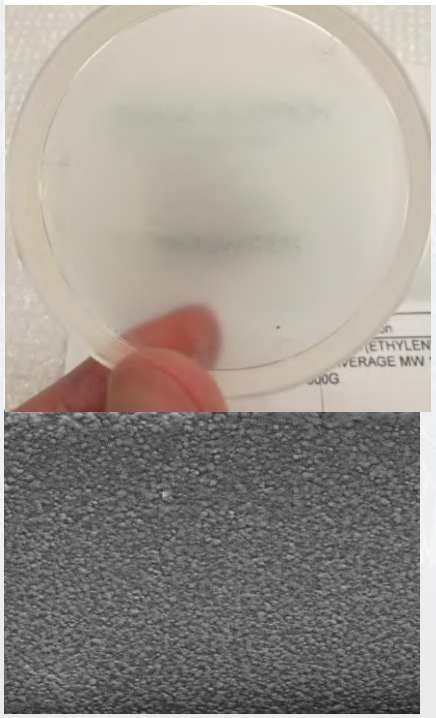
Drop Casting hydrophilic polymer solution with PHX: changing spherulite structure



33% PHX in Biopolymer



33% PHX in Biopolymer blend



- Addition of PHX modifies properties of polymer films
  - Mechanical properties
  - Water and Oxygen permeability
  - Antibacterial/fungal

*University of Guelph: Professor **Loong-Tak Lim**, NSERC Engage*

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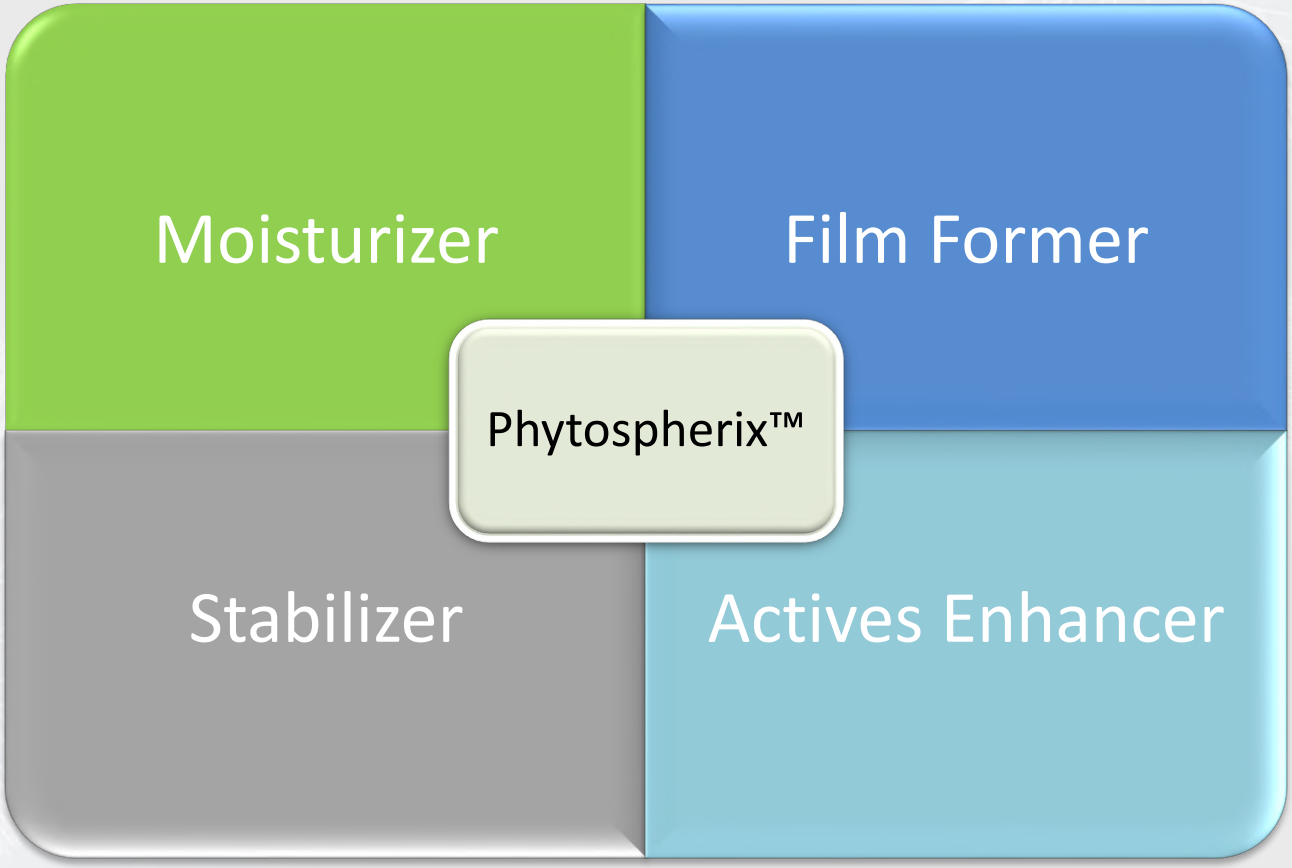
Phytospherix™: Multifunctional Additive



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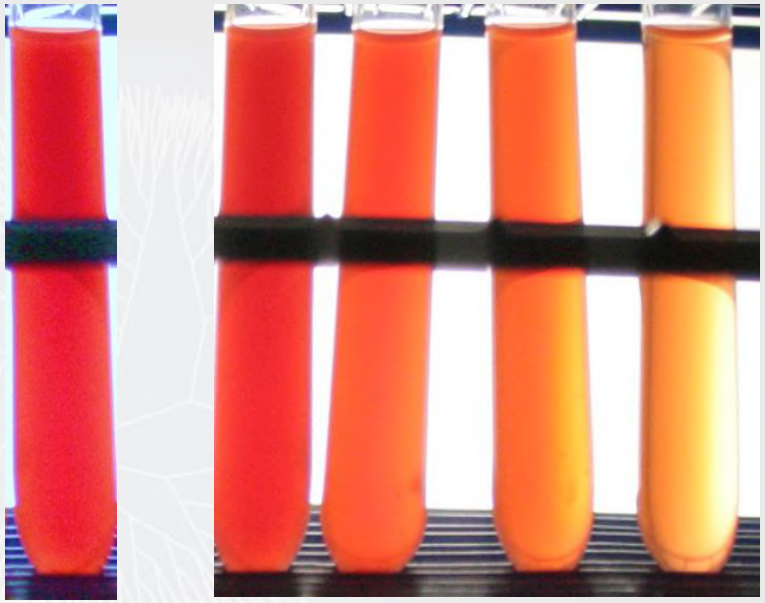
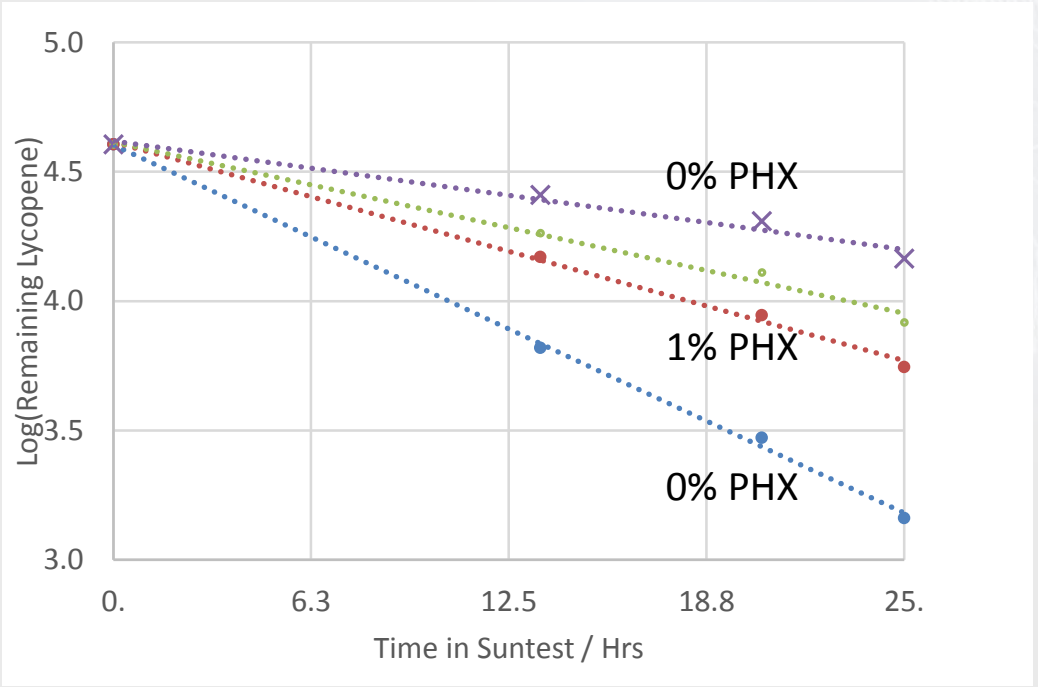
Food/Nutra: Photo-Stabilization of Natural Colors

Adding PHX to formulation increases colour lifetime (and preserves active ingredients)

0 hrs → 250 hrs Sunlight equivalent



0-5% 5% 1% 0.5% 0%



400% increase in half-life

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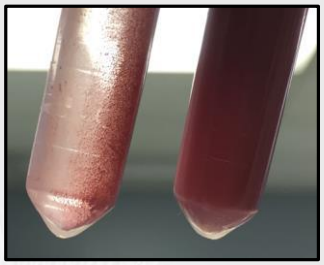
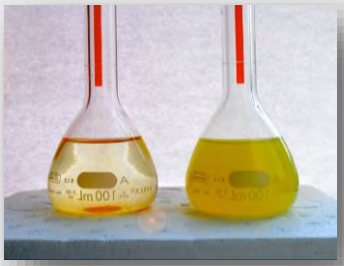
Food/Nutra: Solubilization

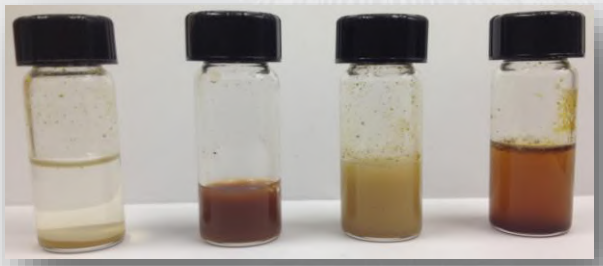
Adding PHX to formulation increases solubility in water



Water +PHX



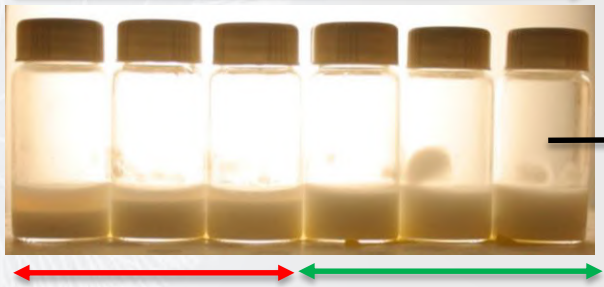
Water +PHX



H_2O modPHX PHX PropGlycol

10% Insoluble Sport Supplement

PHX: 17% 18% 19% 20% 22% 25%



Gel

Unstable Suspension

Stable Gel

PHX: 0% 5% 10% 15% 20%

Solubility → Bioavailability

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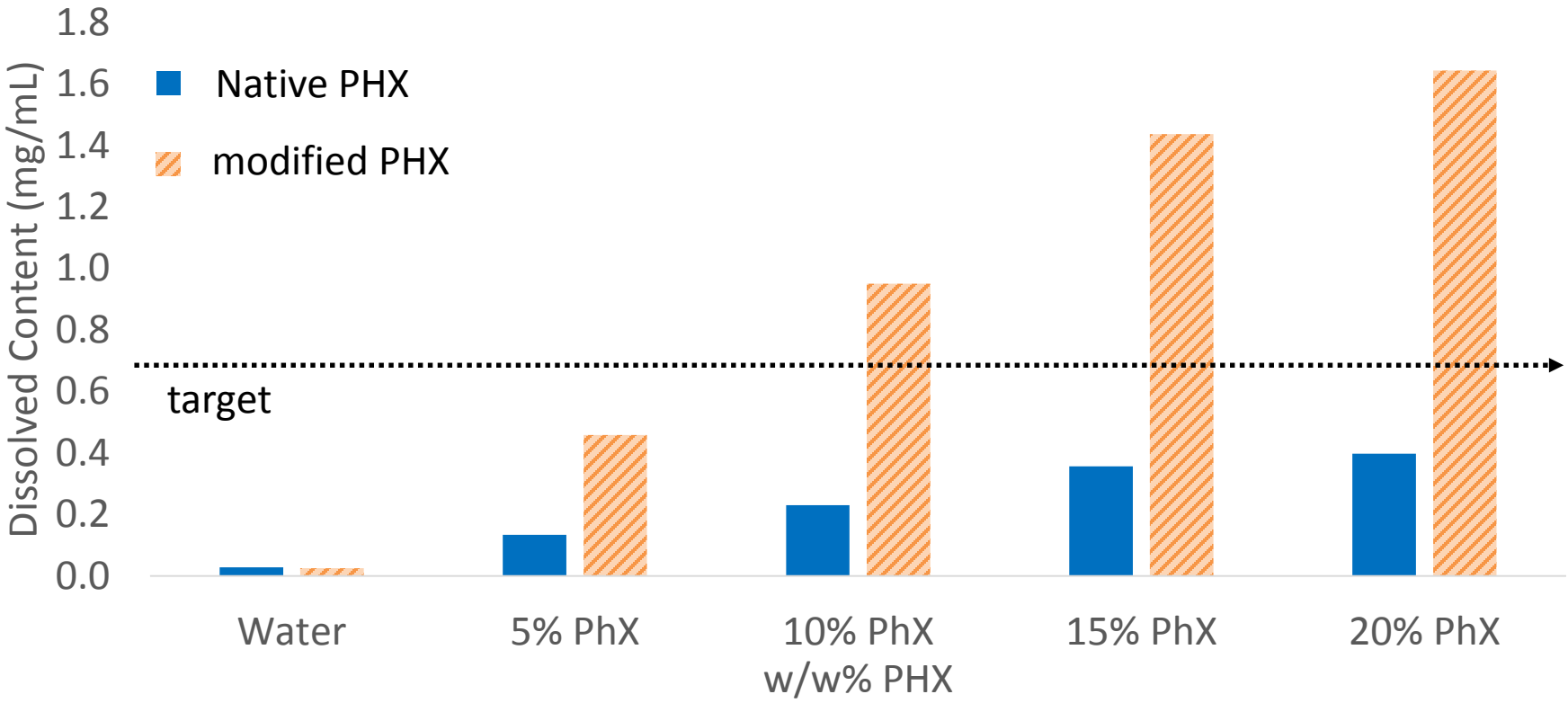
Food/Nutra: Solubilization

Hydrophobic chemical modification can increase solubilizing power for some ingredients



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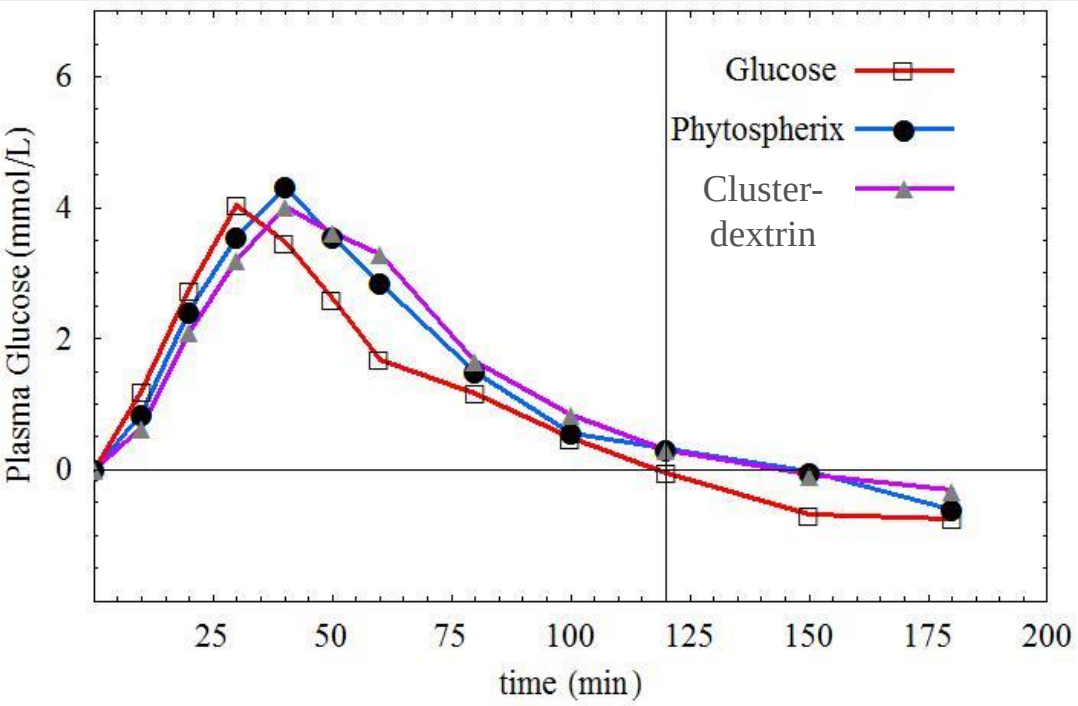
Food/Nutra: Digestibility and Metabolism

- Complete digestion to glucose
  - High **GI** ~0.7-0.9 → Fast digestion
  - Reduced/delayed "sugar crash"

Opportunities in Sports Nutrition



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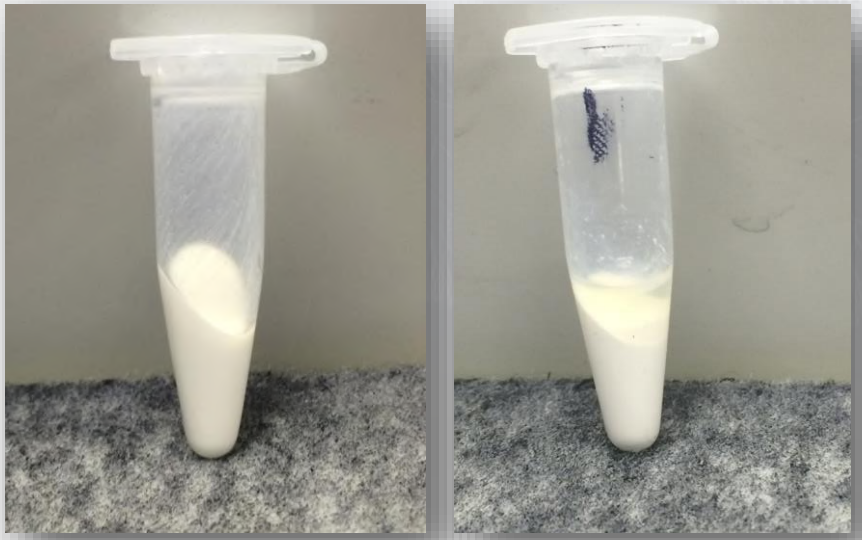
Personal Care: Emulsion Stabilization

Accelerated Stability: 2 months @ 45°C

Emulsion with 0.5% PhytoSpherix™ | Emulsion with 0.5% HA

Emulsion Stability: Rapid Test - Centrifuge

0.5% PhytoSpherix™ | 0.5% HA



No phase separation | Phase separation

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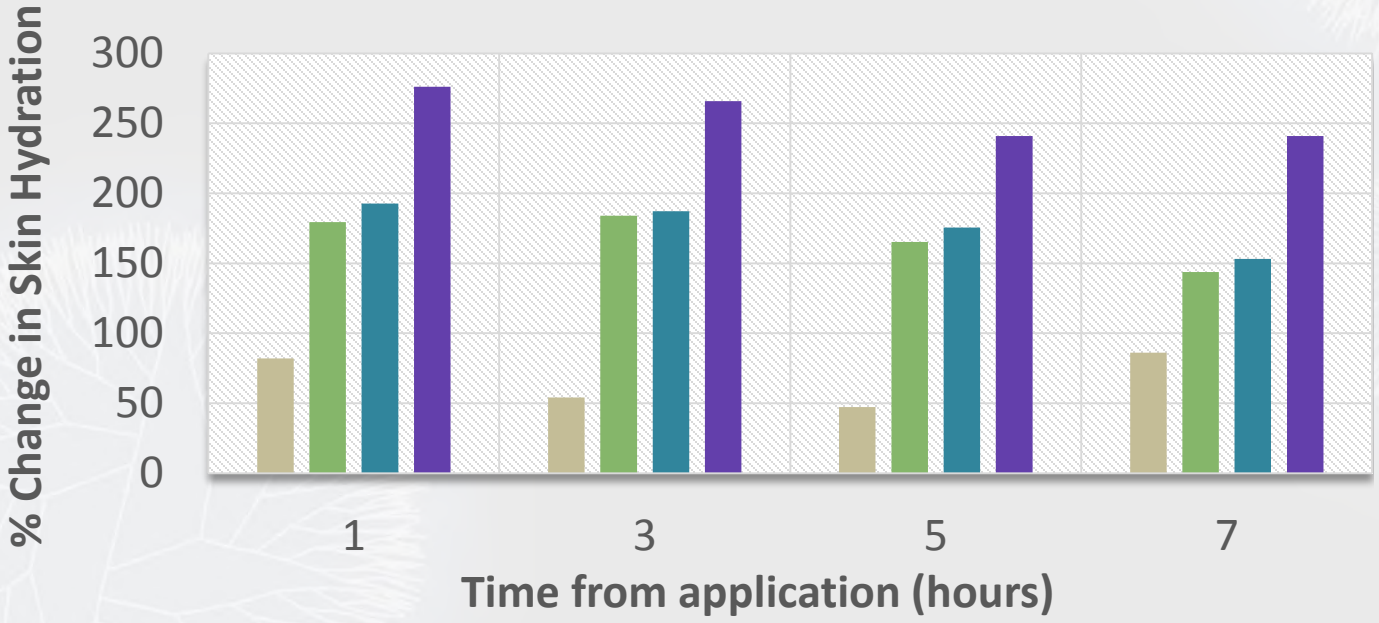
Personal Care: Moisturizer

- **PhytoSpherix™** retains moisture longer than the current best moisturizing agents – outperforms hyaluronic acid



•Gives a long-lasting smooth, velvety feel on the skin

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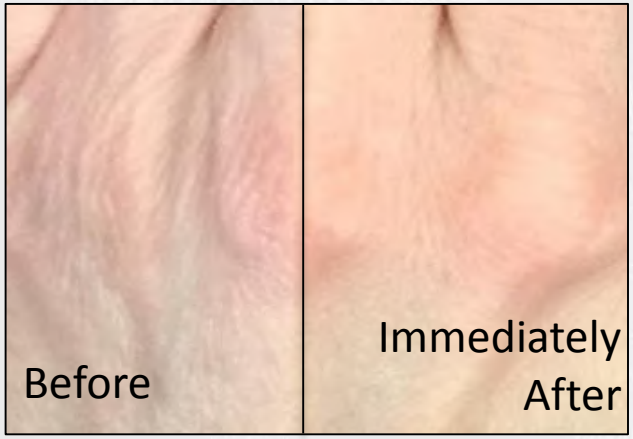
Personal Care: Anti-Aging

Face

Hand



Skin moisturizing lotion formulation

PhytoSpherix™ Increases cell metabolism and natural production of HA and collagen by skin cells

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Pharmaceuticals: Nanomedicine

- To date, Nanomedicine has been disappointing
  - Toxicity of synthetic nanoparticles
  - Lack of water solubility
  - Not stable in blood
  - Particles attacked by immune system
  - Not cleared from the body (biopersistent)
  - Not easy to attach bio-actives
- The **ideal nanoparticle**
  - Safe, non-toxic/allergenic
  - Biodegradable
  - Uniform size & shape
  - Water soluble
  - Easy to modify
  - Evades the immune system

PHX fits the profile

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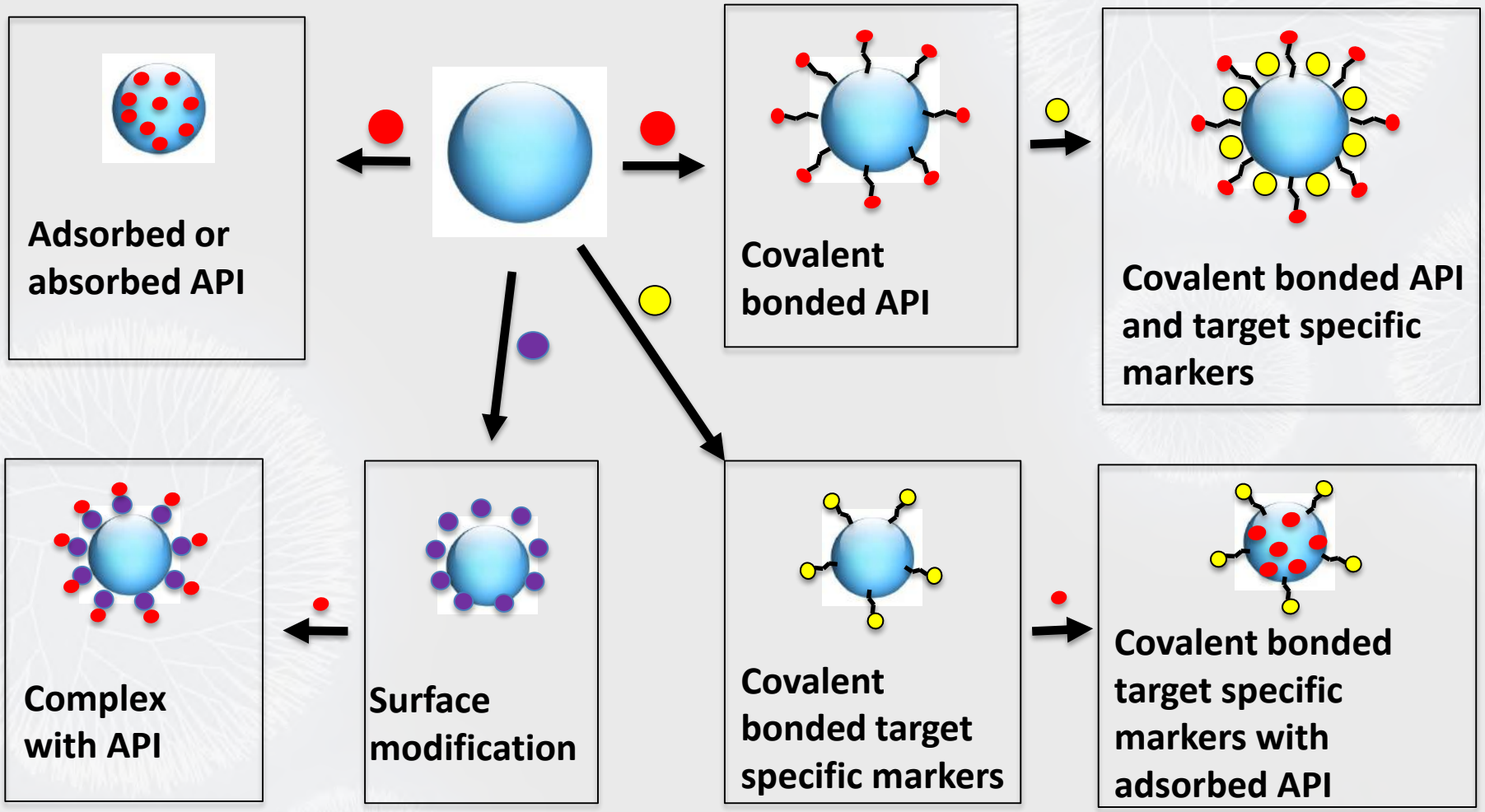
Pharmaceutical: Drug Delivery



PHX (un-modified) is a benign nano-carrier

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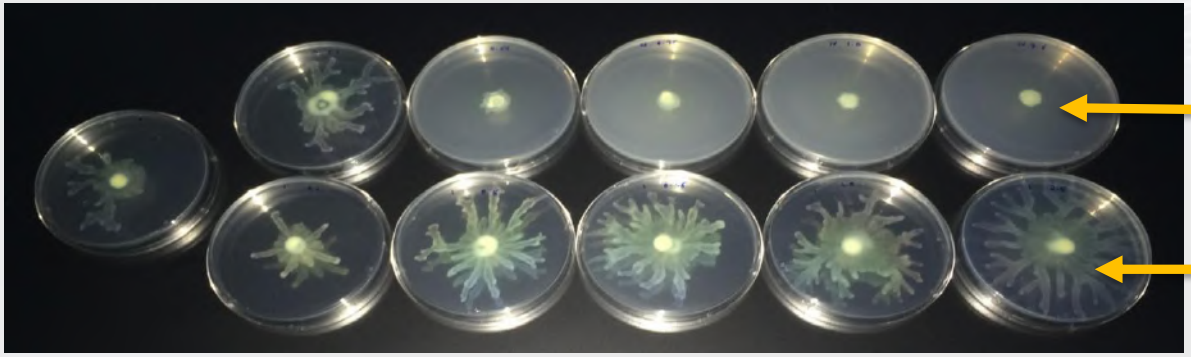
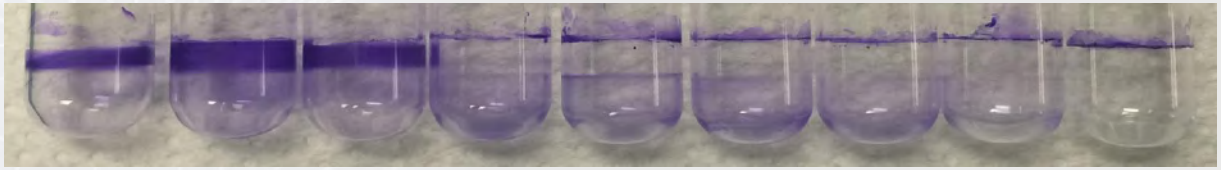
Pharmaceutical: Anti-Biofilm



Adding PHX inhibits biofilm formation



Specific modifications of PHX inhibit biofilm growth and maturation

- Prevents “swarming motility”
- The molecules that are attached to the PHX are not, on their own, effective anti-biofilms – and neither is PHX on its own

*University of Guelph: Professor **Susan Glasauer**, NSERC Engage*

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Pharmaceutical: Antibiotics

Modified PHX Kills Bacteria

P. aeruginosa PAO1

Specific modifications of PHX are effective antimicrobials

- Non-toxic to mammalian cells
- Inhibits growth and/or kills test pathogens
  - The molecules that are attached to the PHX are not, on their own, effective antimicrobials – and neither is PHX on its own
- Nanoparticles shown to concentrate in cells that have been infected
- Inhibits virulence factor production (e.g. pyocyanin)

*University of Guelph: Professor **Susan Glasauer**, NSERC Engage*

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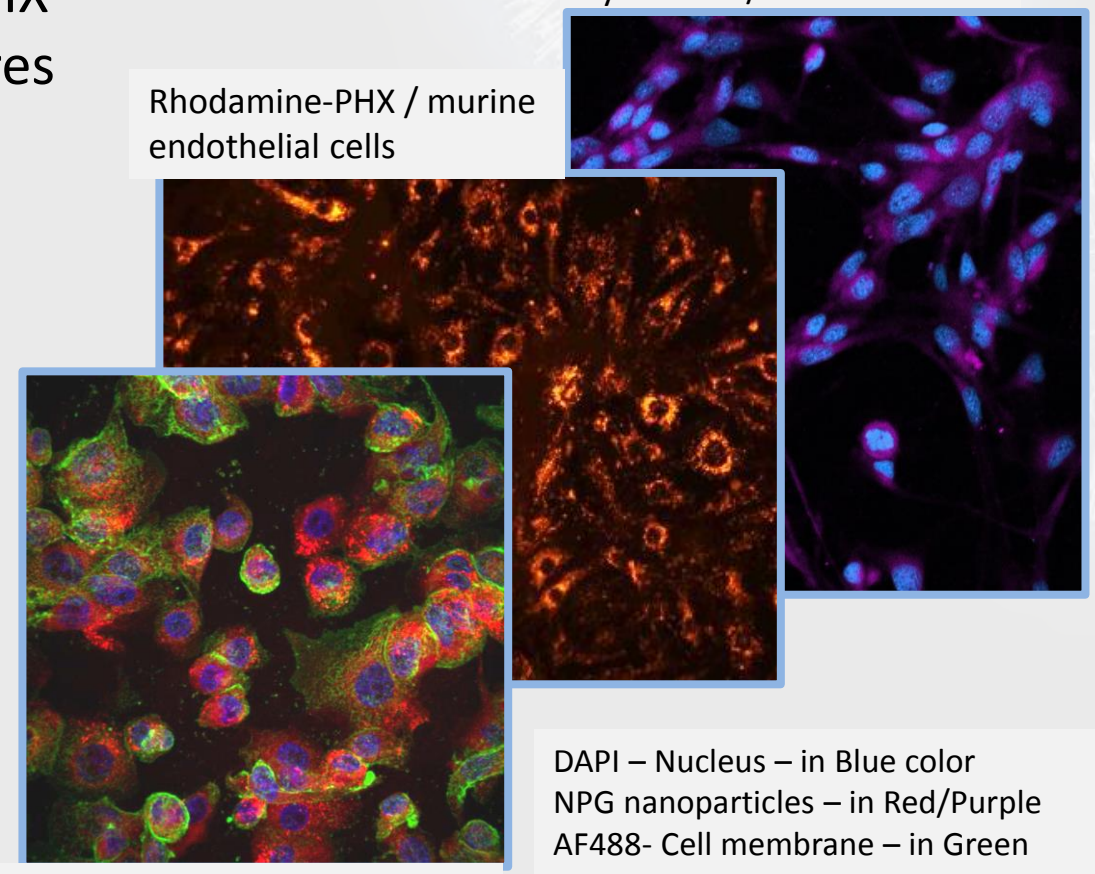
Pharmaceutical: Cellular Uptake

- All cell types internalized PHX with a variety of fluorophores
- Slow uptake: 6-24 hrs
- Accumulated only in cytoplasm

Cy5.5-PHX / fish RTG-2 cells

Rhodamine-PHX / murine endothelial cells



Cy5.5-PHX / human phagocytes

DAPI – Nucleus – in Blue color
NPG nanoparticles – in Red/Purple
AF488- Cell membrane – in Green

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Summary

Natural cosmetics and anti-aging products for the Personal Care market

NanoGlys™

Enhanced nutrition, delivery and athletic performance for the Food and Nutritional Supplement market

Susttain™

Anti-infectives, drug delivery and immunomodulation for the Health Care market

PhytoSpherix™

Looking for R&D Partnerships!

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Conclusions

- Natural nanomaterials are ubiquitous
- Nanomaterials can be safe for humans and the environment
- Nanomaterials can bring functional benefits to food and other edible or topical formulations.
- Natural nanomaterials can open new technological and business opportunities

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Thank you!

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