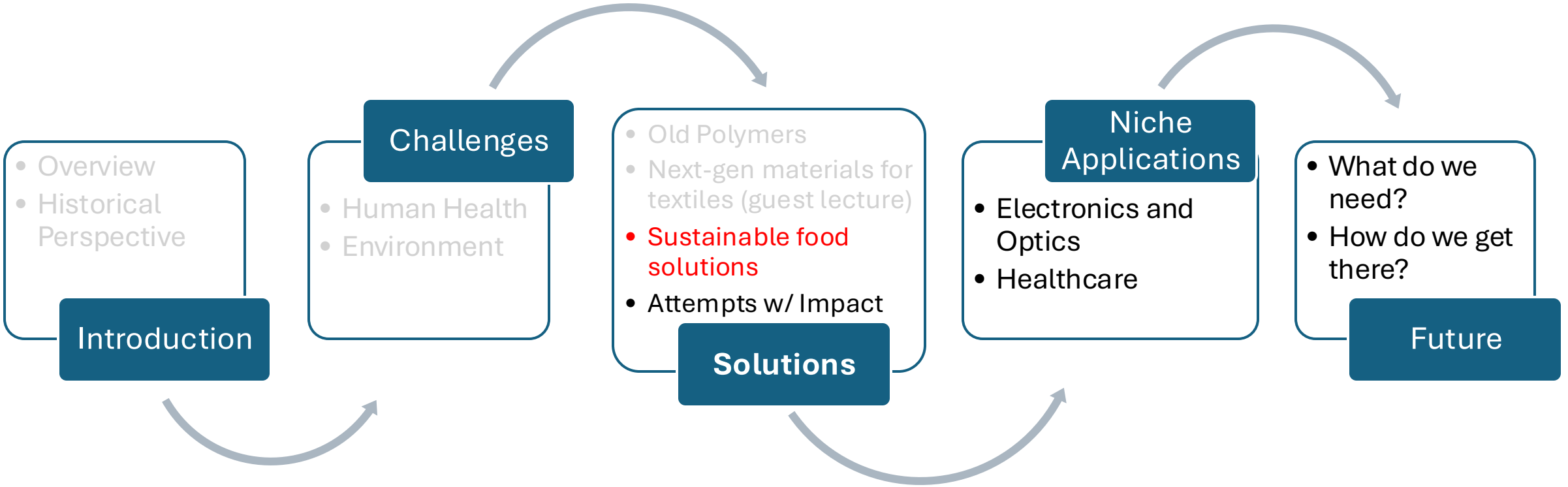
# Sustainable Materials for Food

Sustainable Materials Fall 2024

October 7th, 2024

Lecturer: Lauren Blake, PhD

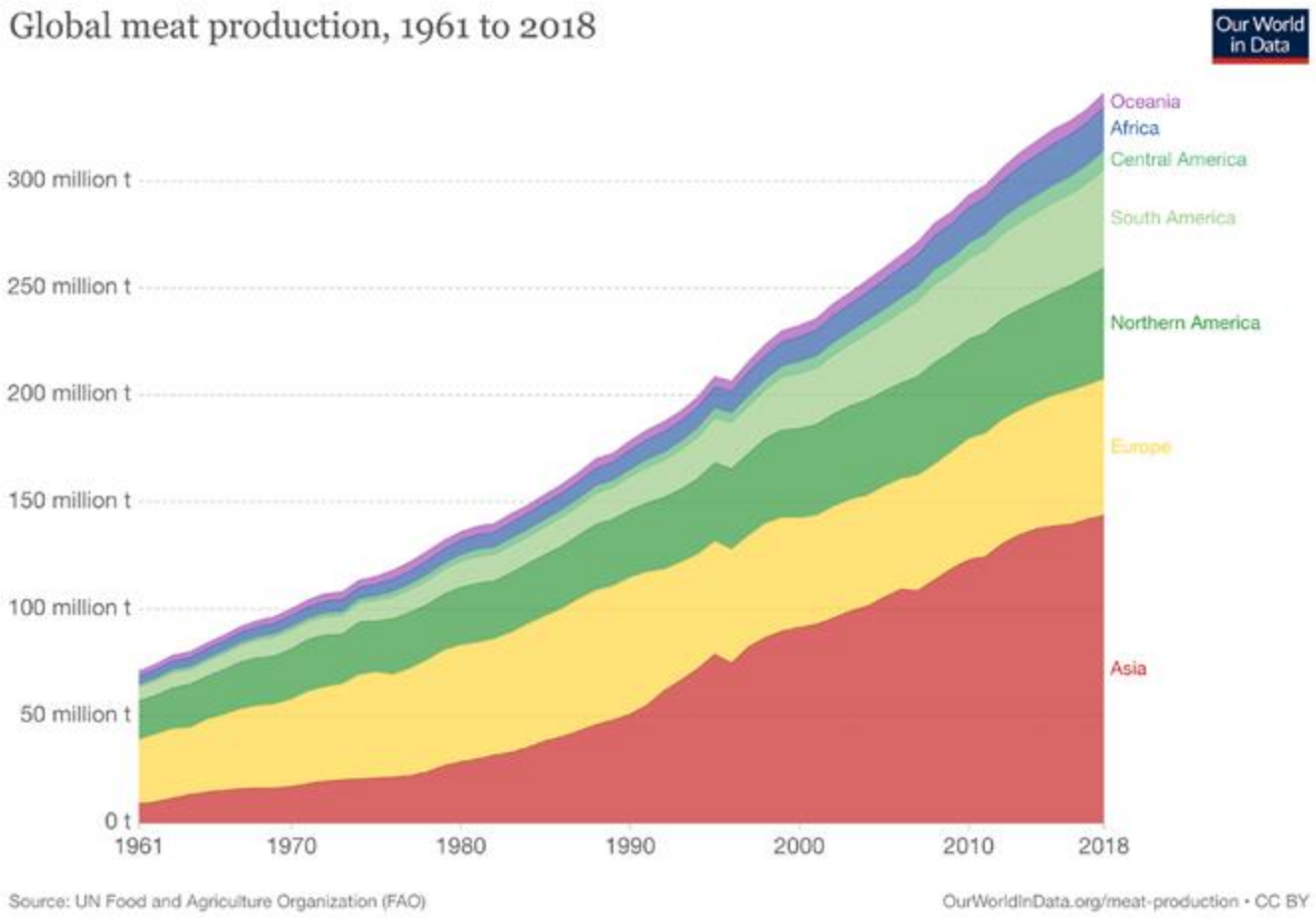
# Lecture 10- (some slight rearrangements)



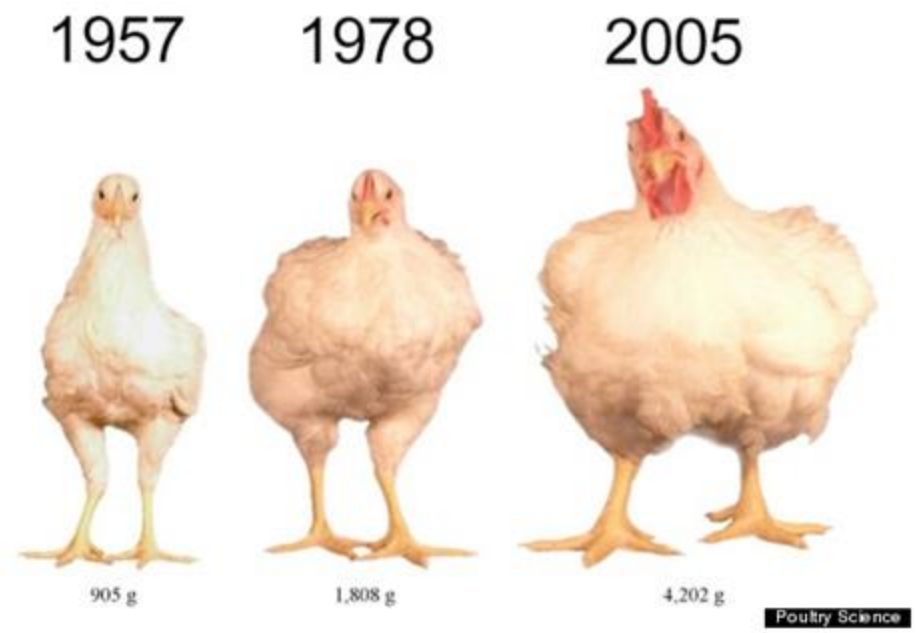
# Learning outcomes

- Get a **broad overview** of the motivation and technical concepts behind alt food
- Understand how the **relevant manufacturing technologies** are applied to food
- Understand the molecular basis for protein texturization
- Know the **components of cell culture** for growing microorganisms as well as stem cells in food applications
- Suggest quality **evaluation methods for alternative proteins**
- Identify several companies in the cellular agriculture field and analyze the biotechnology, motivations, and scientific obstacles behind their products

# Meat demand and production continues to rise

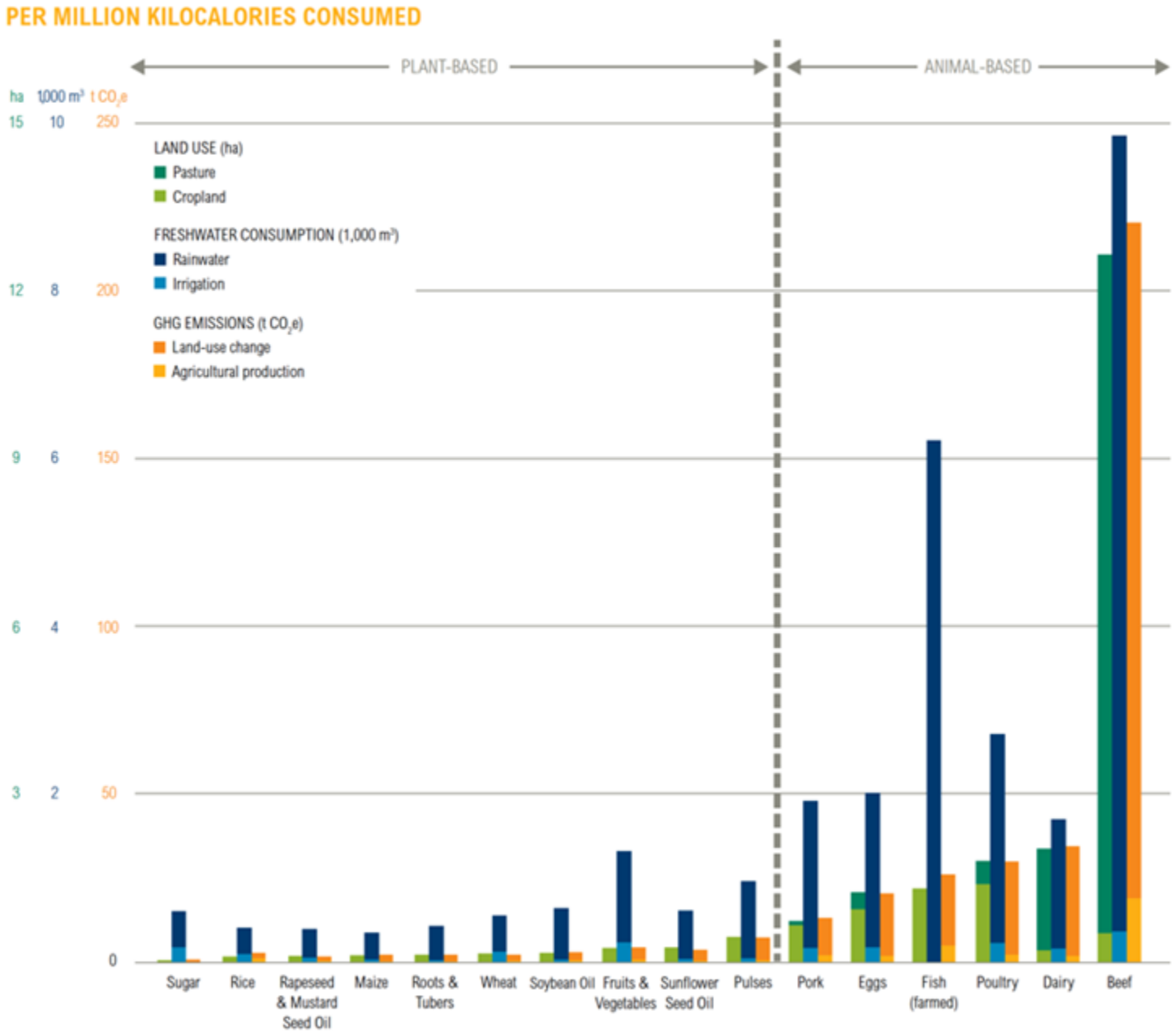



- How are we keeping up?



# Protein from animals is less efficient

- Animal-derived foods are less efficient in their land, water, and energy use as a means to feed this growing population



# There are many issues with current proteins for food

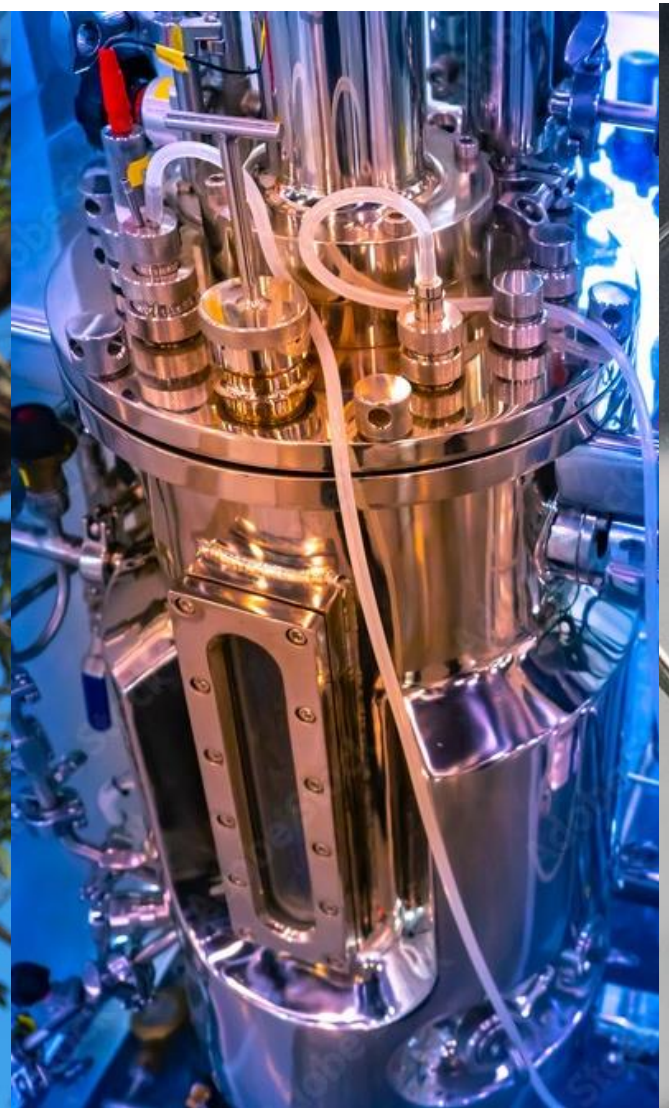
- **Environment**
  - Water and land use
  - Greenhouse gas emission
- **Safety**
  - Increased risk of type 2 diabetes and coronary heart disease
  - Animals in the United States consume more than 2x as many medically important antibiotics as humans do. Medical experts expect 10 million annual deaths from antimicrobial resistance (AMR) in 2050, a 14-fold increase over current deaths.
- **Efficiency**
  - It takes **nine calories** of food fed to a chicken to produce **one calorie** of meat.
  - **75% of agricultural land** is used for raising and feeding livestock yet only provides **1/3** of the global protein supply.
- **Ethics**
  - Animal cruelty

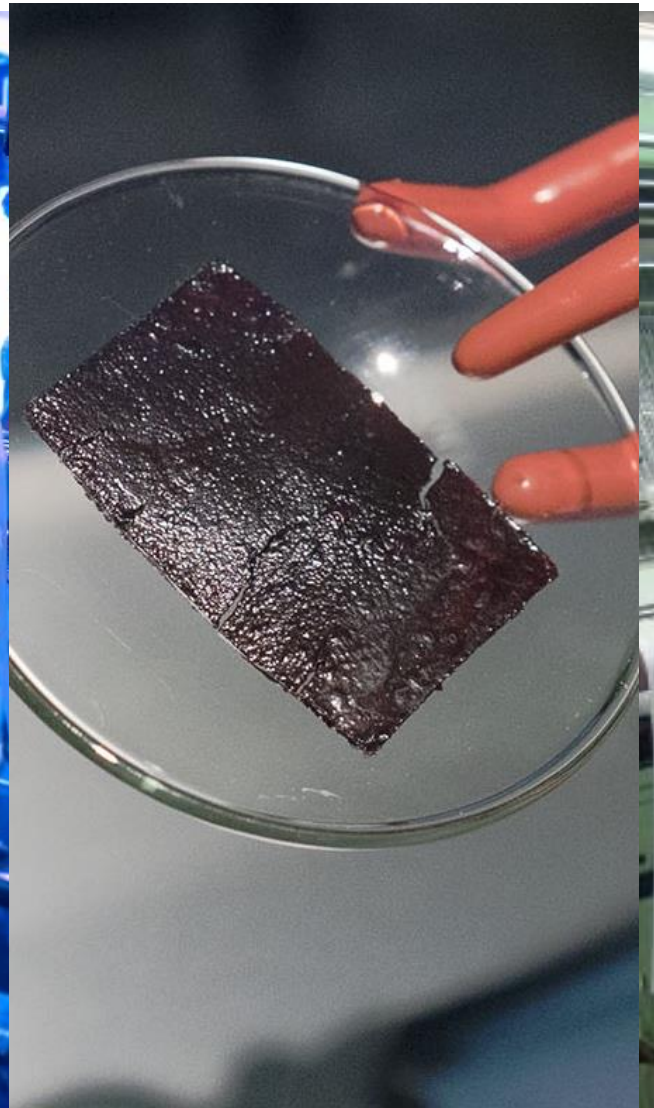
# Different animal proteins are used for different applications

- **Meat**
  - Myofibrillar
  - Sarcoplasmic
  - Stromal
  - Muscle proteins (myofibrillar, sarcoplasmic, and stromal) are used for applications ranging from gelation to color formation
- **Dairy**
  - Casein
  - Whey
  - Casein and whey are used for viscosity and stabilization of various food matrices

- **Egg**
  - Egg white protein is used in forming networks for stability in whipping and heating of food products

# Recall from last week with Sydney Gladman: next-gen materials fall into 4 main categories





# The alternative protein landscape for food is divided into three main pillars

## Plant-based

Photo courtesy of Beyond Meat

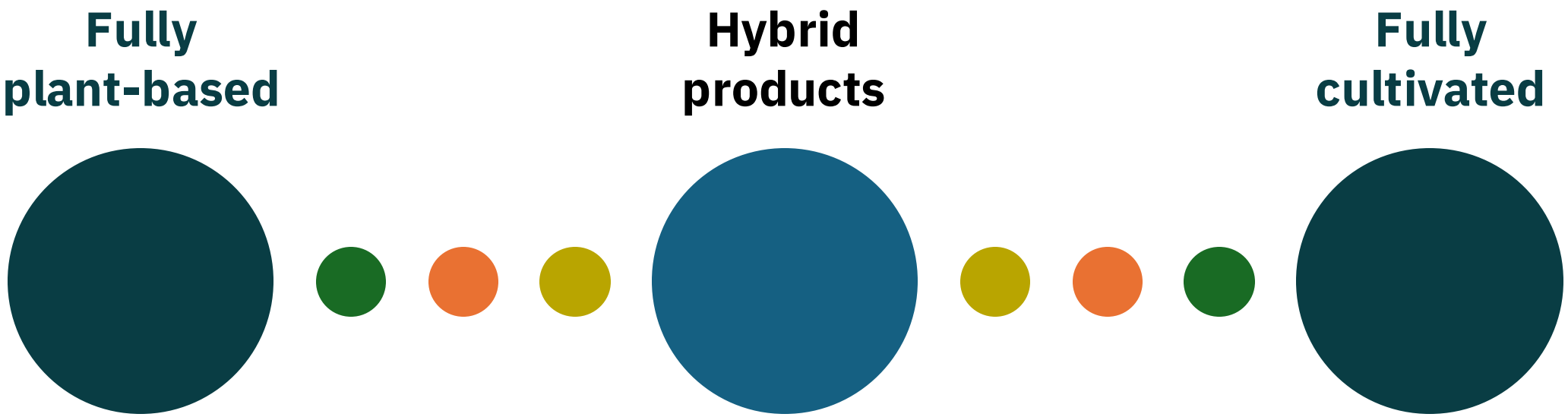
## Fermentation

Photo courtesy of Ecovative Design: Atlast Food Co.

## Cultivated

Photo courtesy of Memphis Meats

# Animal product alternatives exist along a spectrum




*Product Examples*

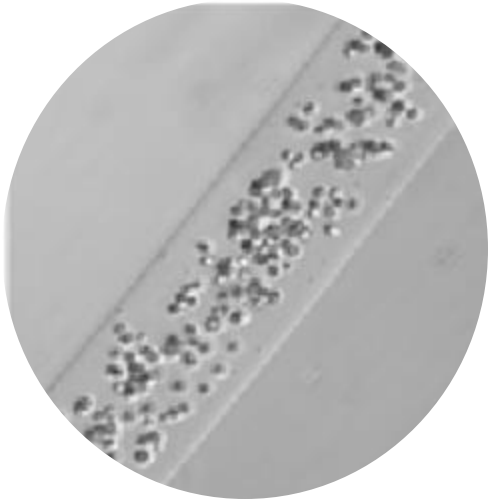
# *Bottom-up approaches* involve assembly of structural elements that are combined

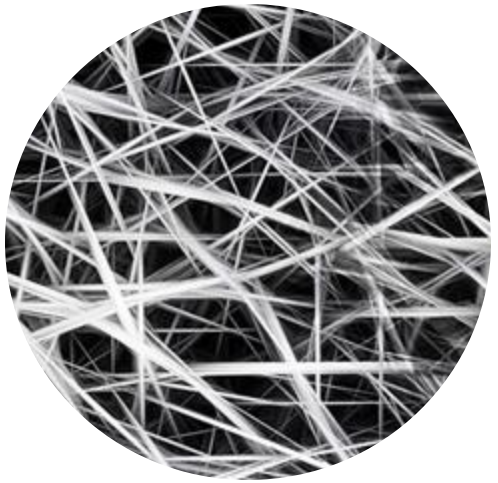
**Mycoprotein**

A fungus (Fusarium venenatum) is produced in a continuous fermentation process in bioreactors.

**Wet spinning**

A solution containing protein is extruded through a spinneret, and subsequently immersed into a bath containing a non-solvent for the protein.



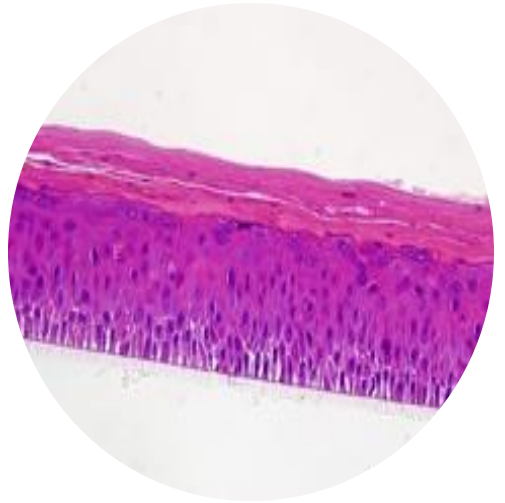


**Electrospinning**

A biopolymer solution is pushed through a hollow needle or spinneret that has an electric potential relative to a ground electrode.

**Cultured meat**

Tissue-engineering techniques are used in vitro to culture animal cells. One exemplary burger has been fabricated using this process.



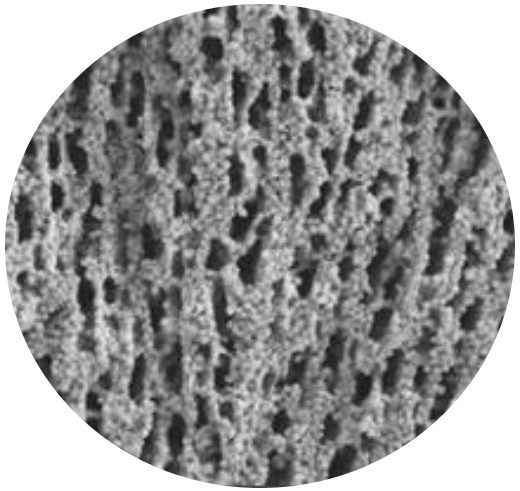
# *Top-down approach* refers to structuring of biopolymer blends using an overall force field

**Extrusion**

Plant proteins are plasticized/molten by a combination of heating, hydration and mechanical deformation.

**Mixing of proteins and hydrocolloids**

Mixing protein with hydrocolloids that precipitate with multivalent cations yields fibrous products



**Freeze structuring**

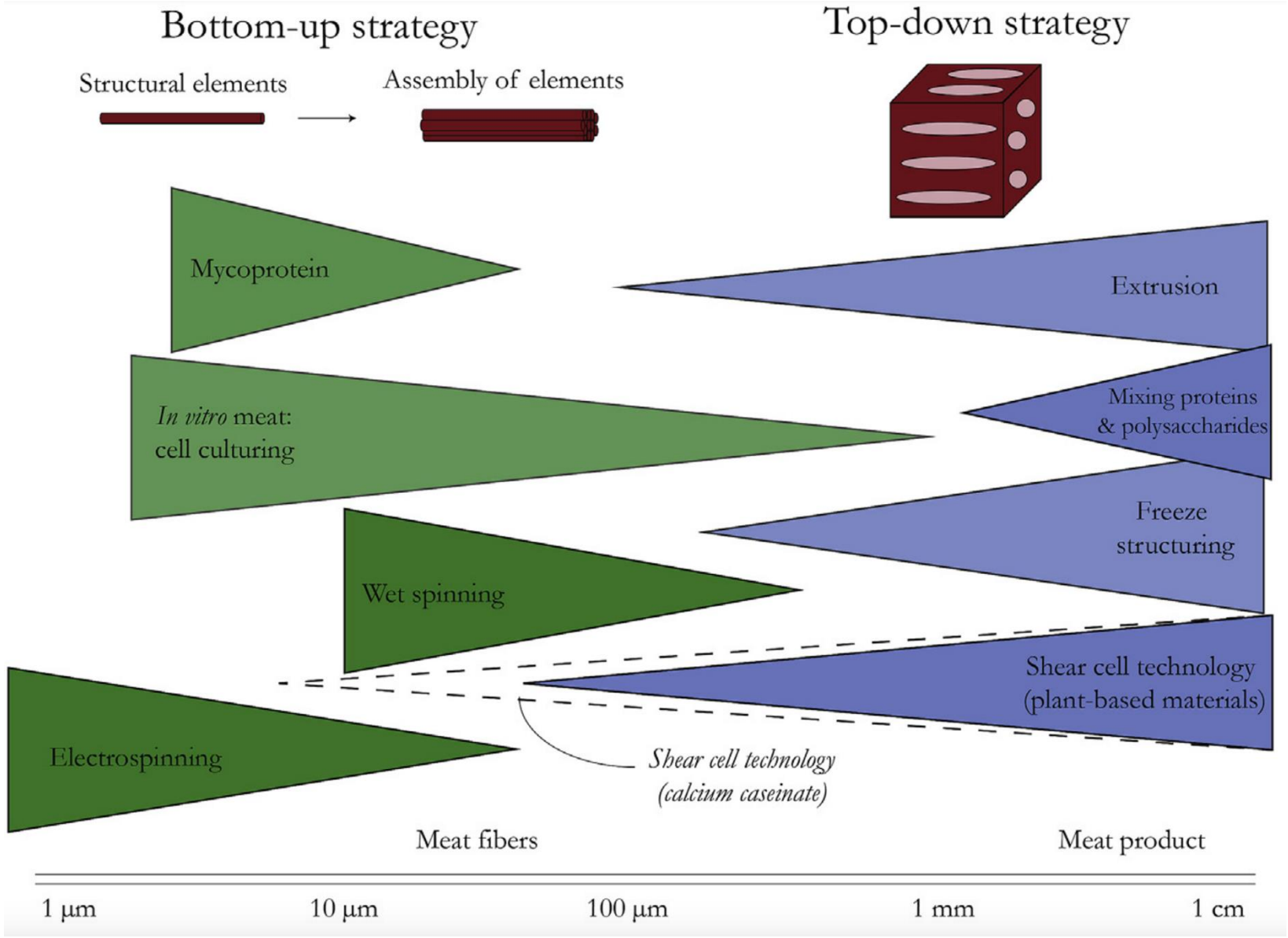
A slurry of proteins is frozen to generate structure

**Shear cell technology**

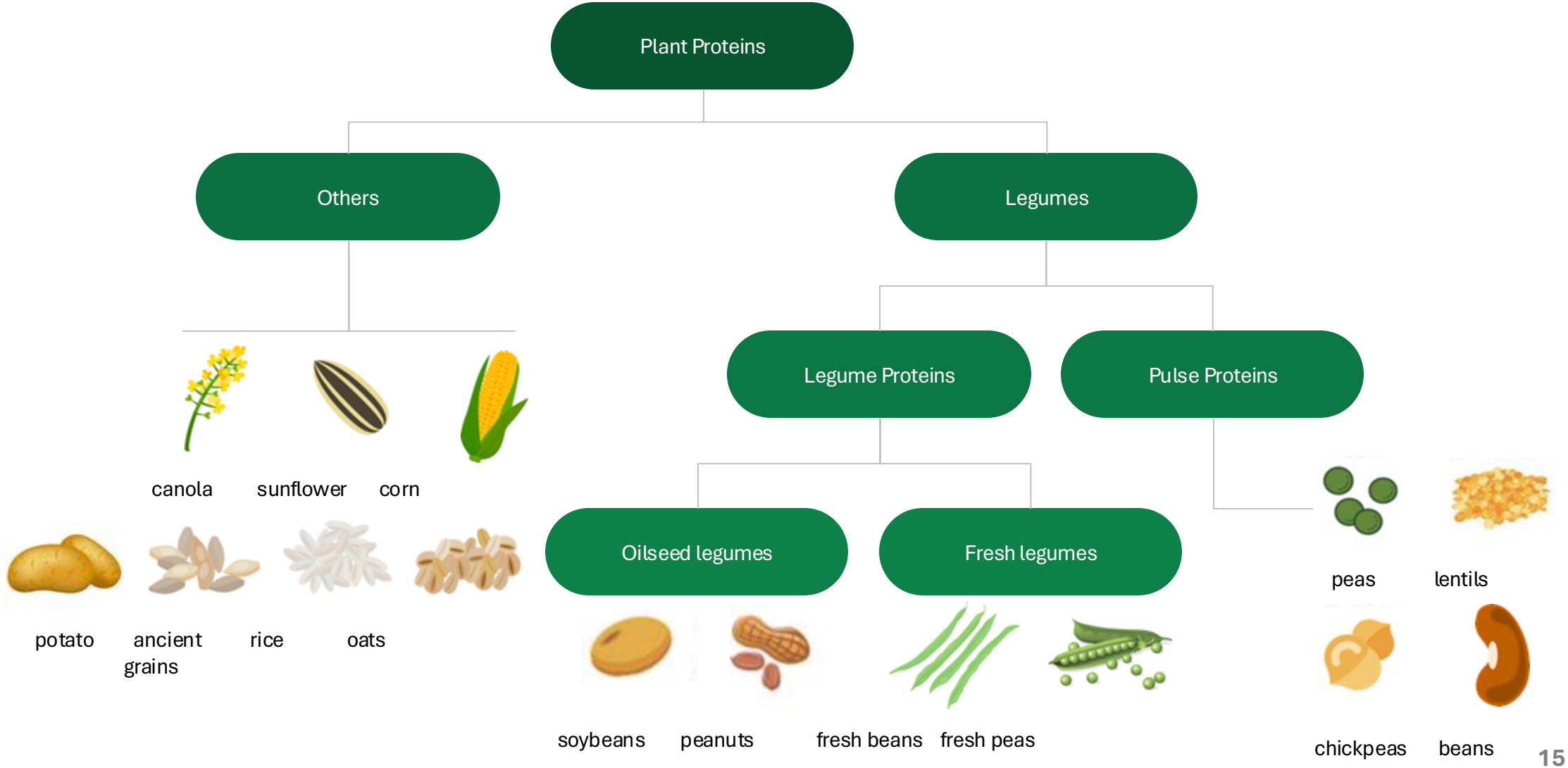
Shearing devices inspired by rheometers, the so-called shear cells, were developed in which intensive shear can be applied.

# Discussion question:

## What are the merits of top-down approaches versus bottom-up approaches?



# All plants have protein, just different amounts and ratios of amino acids



# Plant protein products exist on a processing spectrum

Less processed


More processed

Beans, lentils, mushrooms, etc.

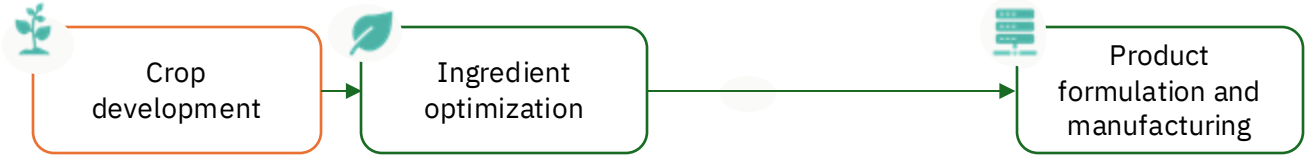
Tofu, tempeh, seitan, etc.

Bean burgers

Meat mimics

# Plant-based meat alternatives are ripe for innovation

- **Core research areas**




- End product

# Texturization of protein turns it from powder to fibrous structure

Texturization of proteins is the process of creating a 3-dimensional structure to match the **texture, mouthfeel, and appearance** of animal-based meat

- Denatures and aligns proteins
- Forms fibers that resemble muscle tissue
- Produces end products such as granules, shreds, and chunks

# There are many ways to texturize plant protein

EXISTING

- Mixing, molding, forming
- Twin screw high moisture extrusion
- Low moisture extrusion

MECHANICAL INNOVATION

- 3D printing & electrospinning
- Shear cell technology

BIOLOGICAL INNOVATION

- Enzymatic functionalization & texturization
- Fermentation
- Tissue Engineering

# Extrusion cooking is the most common texturizing technology

Extrusion transforms **native ingredient biopolymers (inputs) into a continuous semi-solid (output)**. To complete this process, a screw system within a barrel conveys mass (a combination of dry ingredients, water, and/or oil) through a die (small opening).

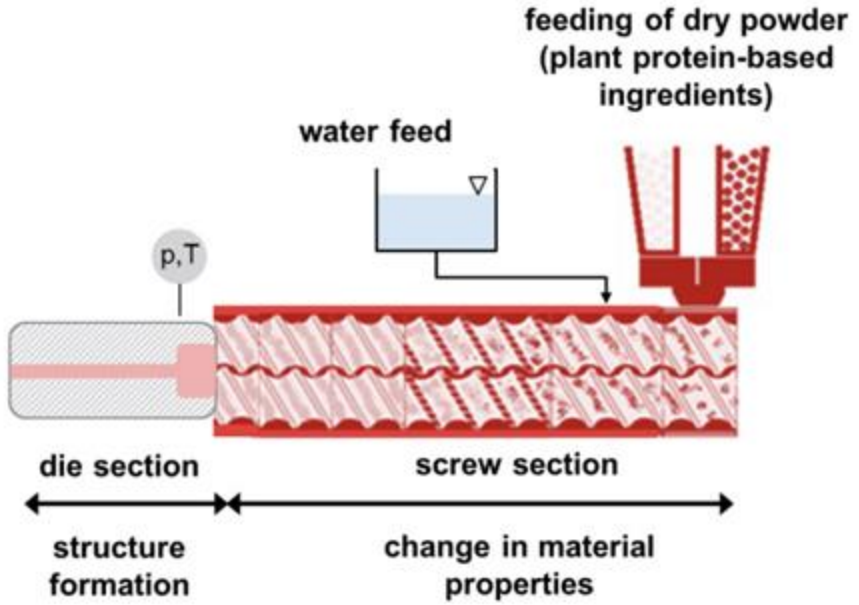
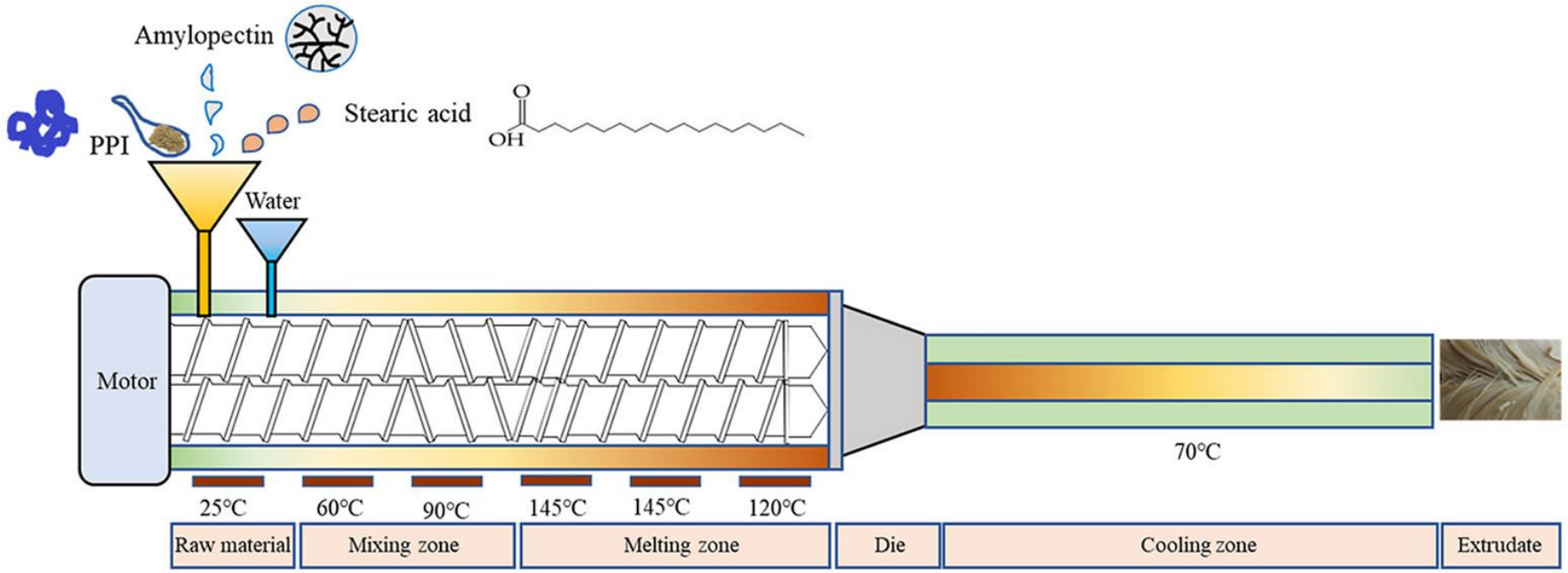
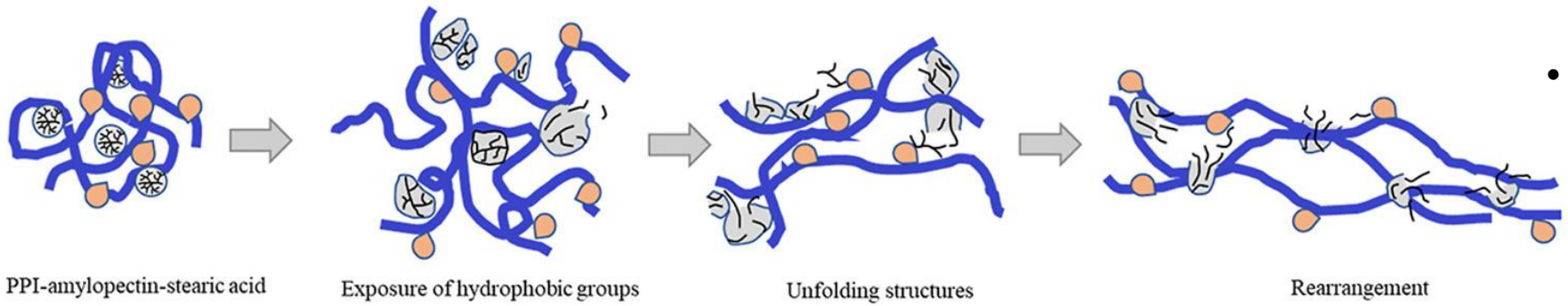



Figure 2: Schematic setup of the Process 11 Hygienic Twin-screw Extruder with length to diameter ratio of 40 and cooled slit die.

Source: Pietsch et al. ThermoFisher Whitepaper, **2021**.

# Texturization involves denaturing proteins and reforming new bonds



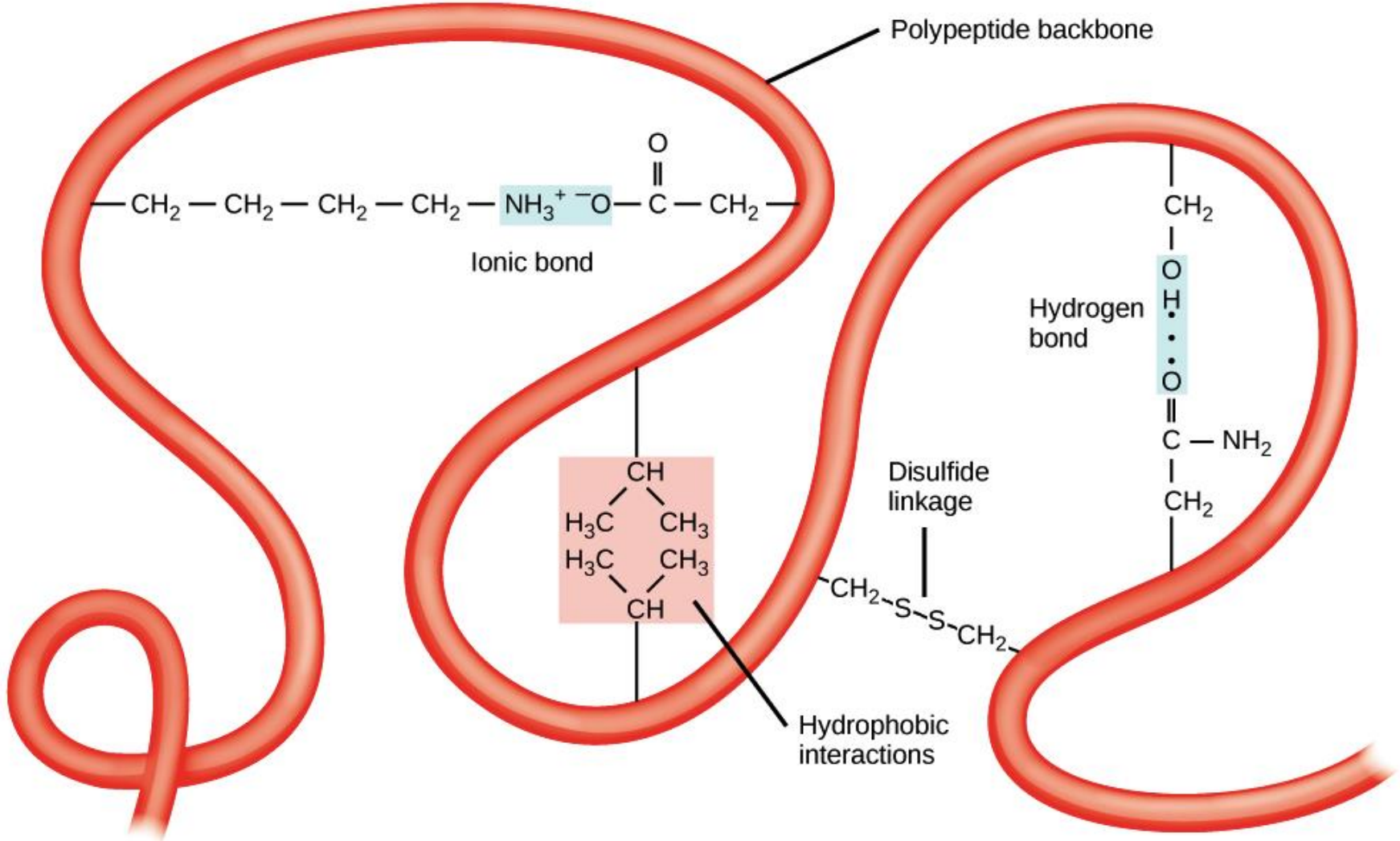




**Discussion question:**
**What kinds of bonds are re-forming in the extrudate?**

**Possible answers:**

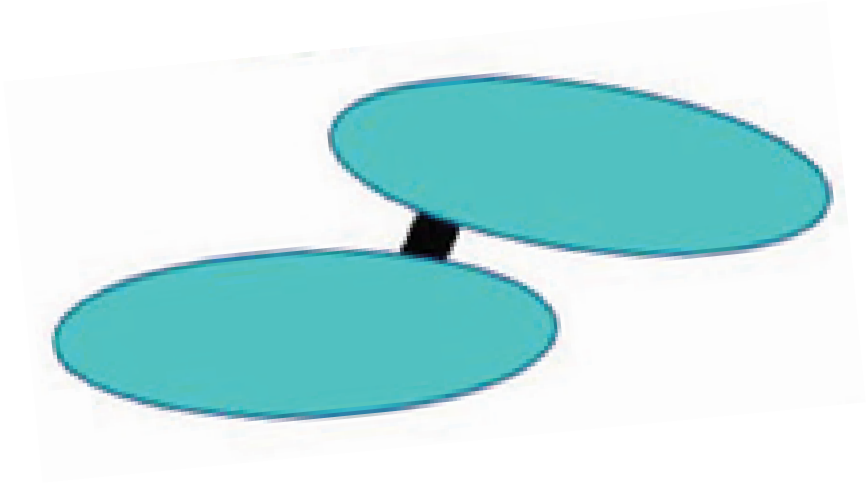
- Hydrophobic interactions
- Hydrogen bonds
- Ionic interactions between charged amino acids
- COVALENT disulfide bonds (if protein contains cysteines)
- Van der waals

# Many types of molecular interactions occur to texturize proteins

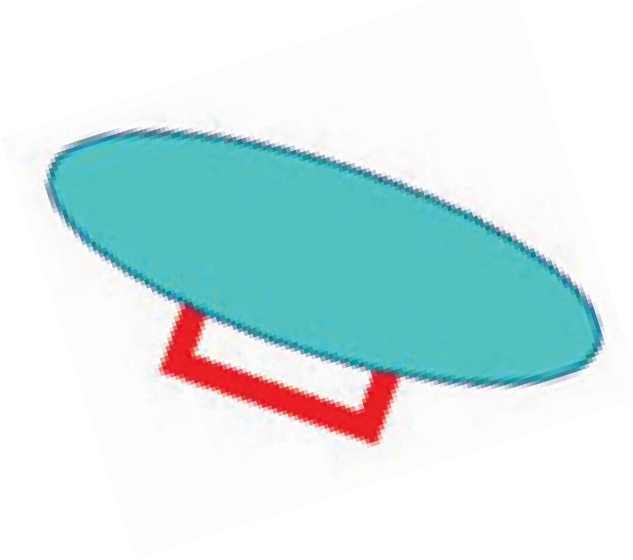


# Discussion: Are INTER-molecular or INTRA-molecular interactions preferred during texturization processes?

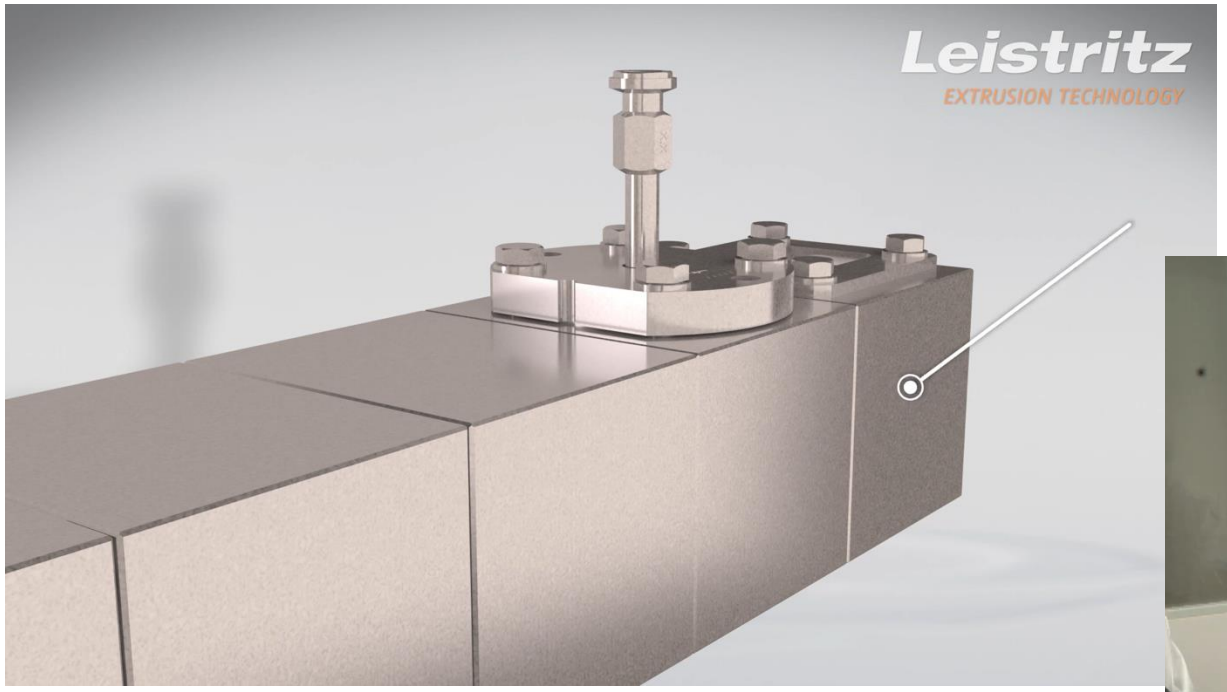
**INTERmolecular:** between DIFFERENT strands



**INTRAmolecular:** within the SAME strand



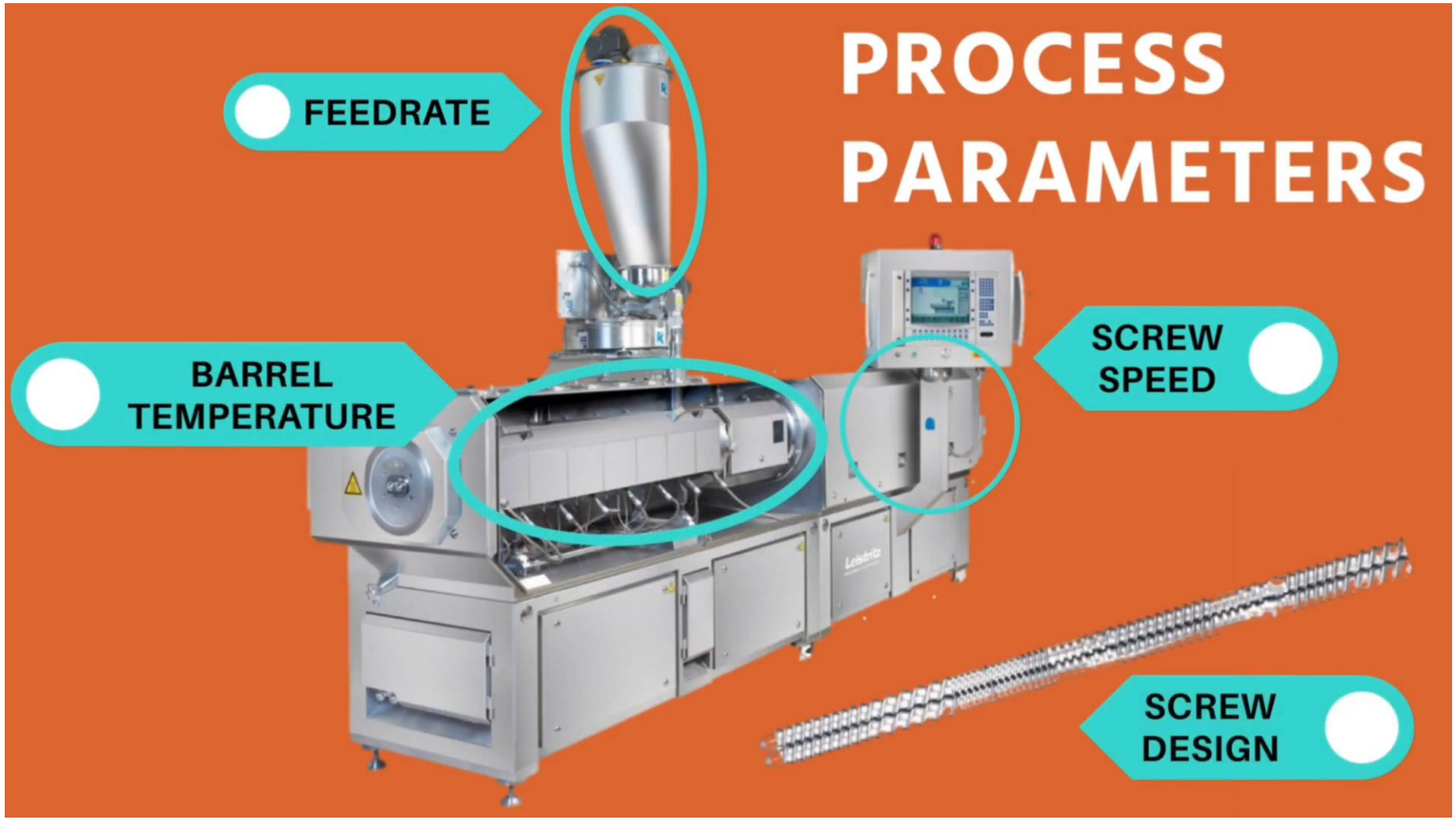
# Extruders are heavy duty equipment





Source: https://www.youtube.com/watch?v=HP8Y7faobqI

# Parameters and screw design make a big difference in optimization



# Low- vs. high-moisture extrusion results in different food applications

## LOW-MOISTURE EXTRUDATE

- Extrusion inlet/process moisture content < 35%
- Restructured meat products (e.g., patties and links); made by hydrating TVP in water or broth (may also include oils)
- Hydrated product is blended with other ingredients to impart the taste, texture, color, and aroma of meat

## HIGH-MOISTURE EXTRUDATE

- Extrusion inlet/process moisture content > 50%
- Whole muscle type products; best resembles muscle striata (e.g., fillets and strips)
- Extruded in a high-moisture form, then passed through one or more steps including marinating, coating, and cooling (refrigeration/freezing)

# Production technology landscape

EXISTING

Mixing, molding, forming

Twin screw high moisture extrusion

Low moisture extrusion

MECHANICAL INNOVATION

3D printing & electrospinning

Shear cell technology

BIOLOGICAL INNOVATION

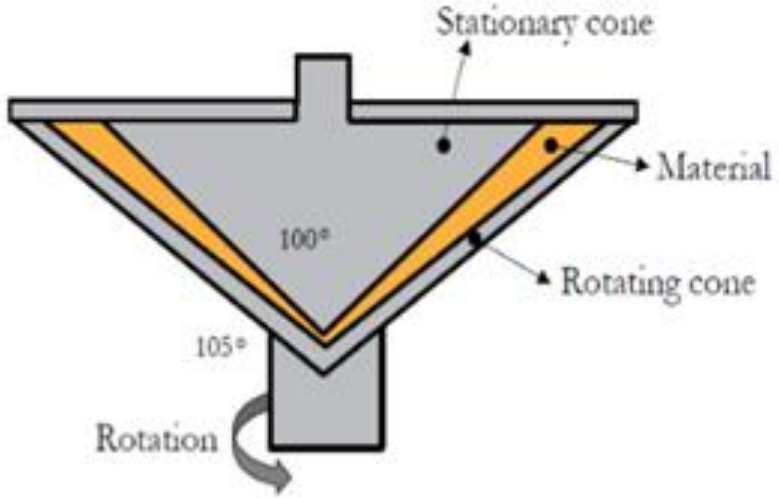
Enzymatic functionalization & texturization

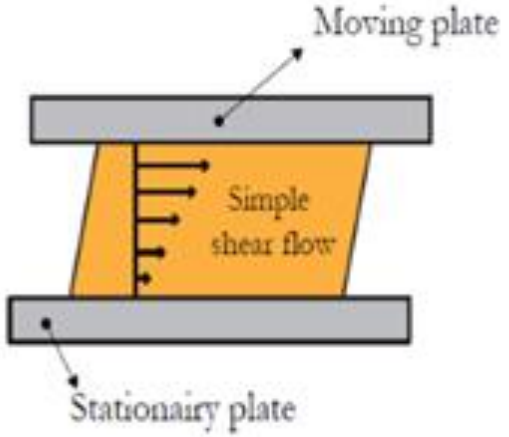
Fermentation

Tissue Engineering

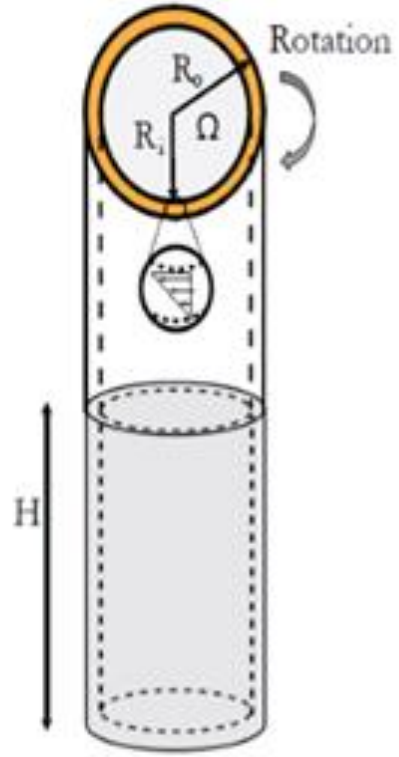
# Emerging tech: shear cell technology can use shear forces to texturize plant proteins


Conical shear cell




Couete shear cell




Source: Birgit L. Dekkers Creation of fibrous plant protein foods. PhD thesis, Wageningen University, Wageningen, the Netherlands (2018). ISBN 978-94-6343-319-8

# Shear cell technology is gaining popularity


Source: https://www.youtube.com/watch?v=HP8Y7faobqI

# Shear cell technology produce very fibrous protein materials for alternative meats


calcium caseinate


soy protein isolate-wheat gluten blend


soy protein concentrate

## Advantages

- Decently robust
- Decently scalable
- Lower environmental impact

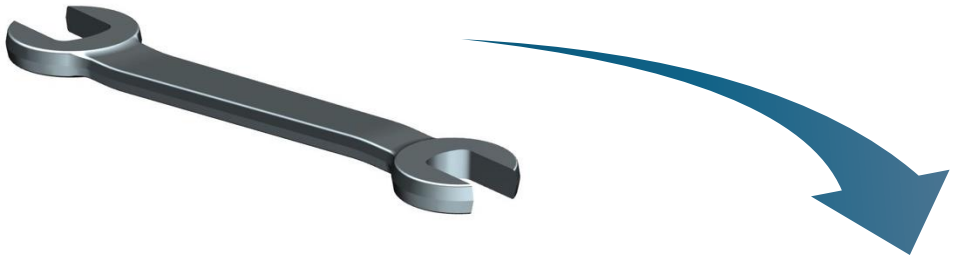
## Disadvantages

- Less commercial availability
- Orientation on microscale only

Source: Birgit L. Dekkers Creation of fibrous plant protein foods. PhD thesis, Wageningen University, Wageningen, the Netherlands (2018). ISBN 978-94-6343-319-8

# Additive manufacturing involves building materials one layer at a time, ideal for structured materials

Digital Design-to-Fabrication



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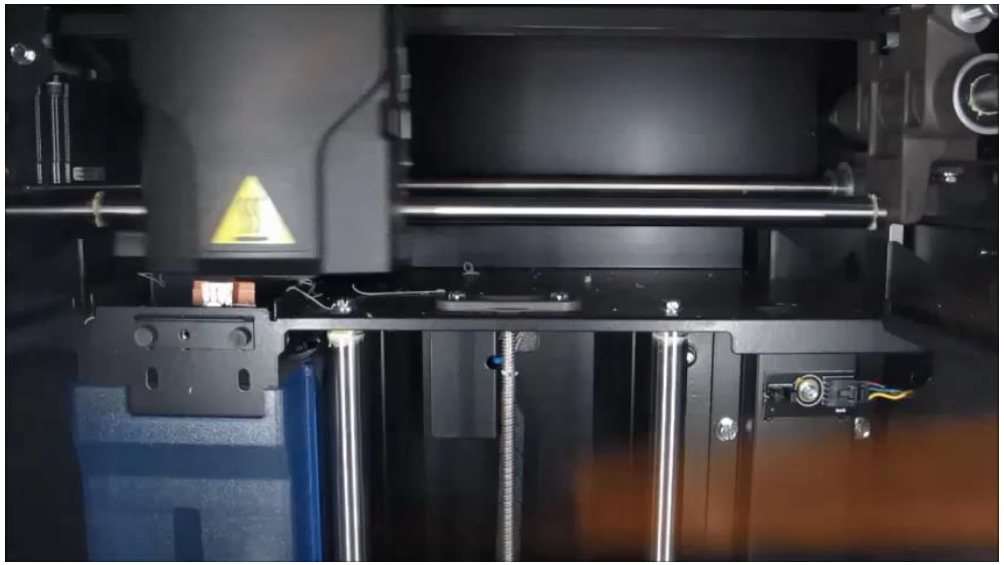


**No custom programming! vs. CNC**

source://german.injectionmouldtooling.com

**No molds! vs. injection molding, stamping, casting, etc.**

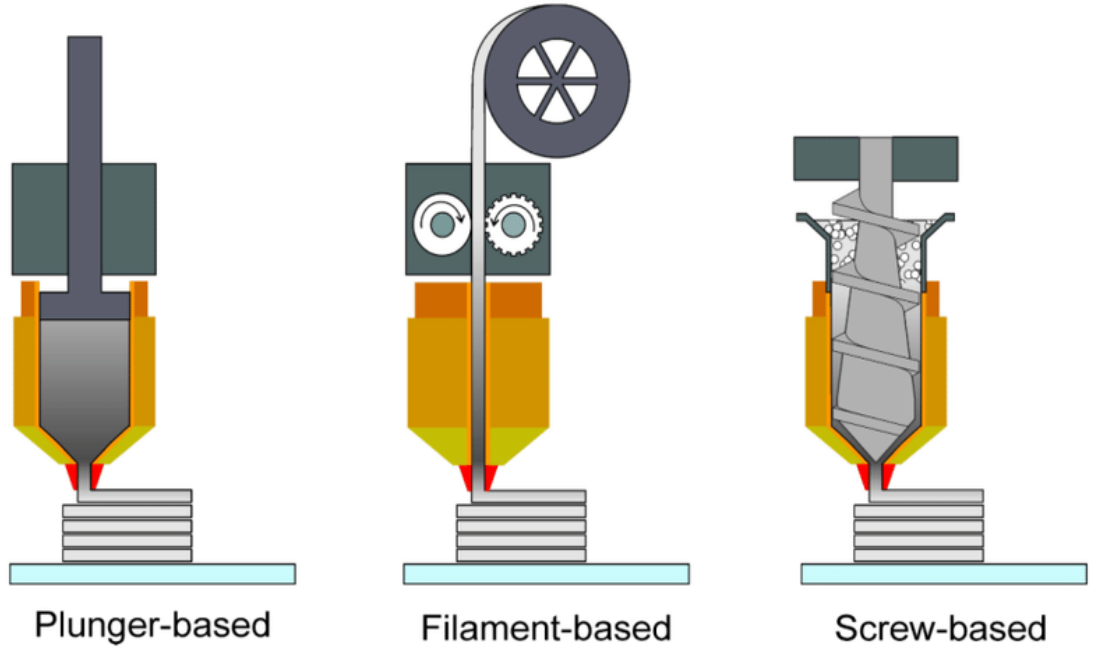
# Additive Manufacturing (AM) has many advantages

- **Shape complexity:** It is possible to build virtually any shape.
- **Material complexity:** Material can be processed one point, or one layer, at a time as a single material or as a combination of materials.
- **Hierarchical complexity:** Features can be designed with shape complexity across multiple size scales.
- **Functional complexity:** Functional devices can be produced in one build.

Credit: Redefine meat 3D printed steak

# Additive manufacturing can also be done by material extrusion

- The build material is pushed through a nozzle in a continuous stream
- A printhead, containing the nozzle, moves around for layer-by-layer deposition
- Deposited material hardens and retains its shape
- The next layer is added on top, and the process is repeated
- Two basic types:
  1. Fused Deposition Modeling (FDM) and
  2. Direct Ink Writing (DIW)



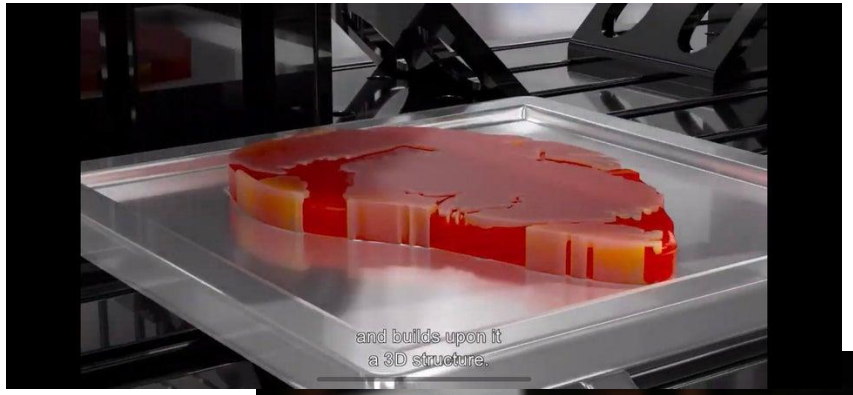

**Material extrusion techniques**

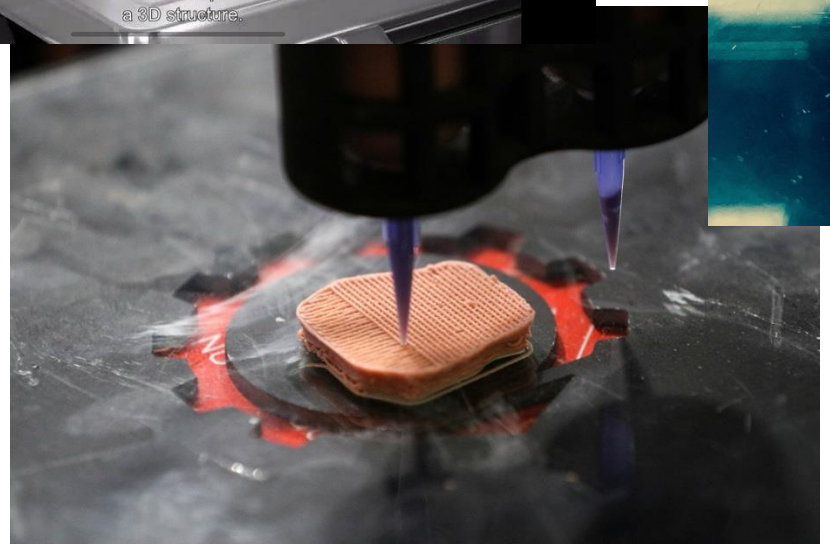


DOI:10.13140/RG.2.2.23981.54248

# Several startups are making structured meats using 3D printing technologies

Credit: MeaTech



Credit: Novameat

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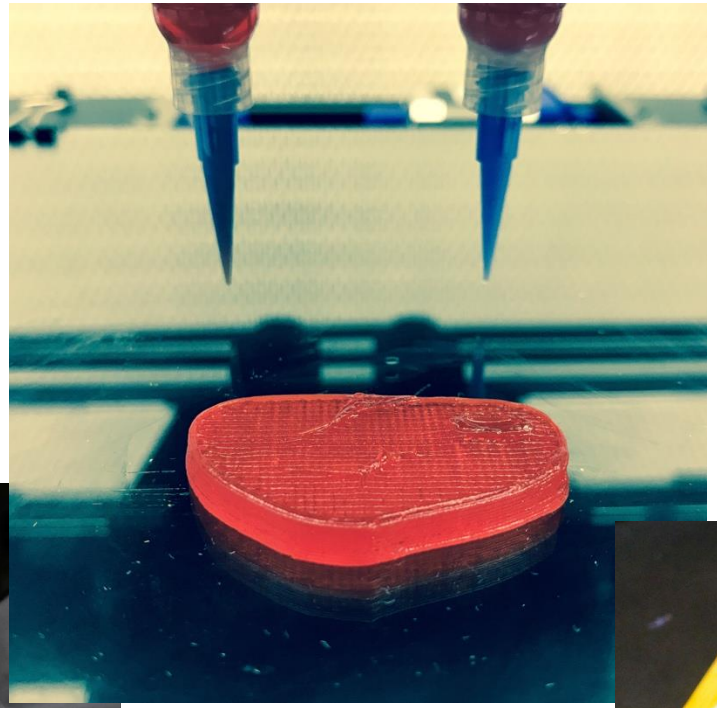


Credit: Cocuus

# Production technology landscape

EXISTING

- Mixing, molding, forming
- Twin screw high moisture extrusion
- Low moisture extrusion

MECHANICAL INNOVATION

- 3D printing & electrospinning
- Shear cell technology

BIOLOGICAL INNOVATION

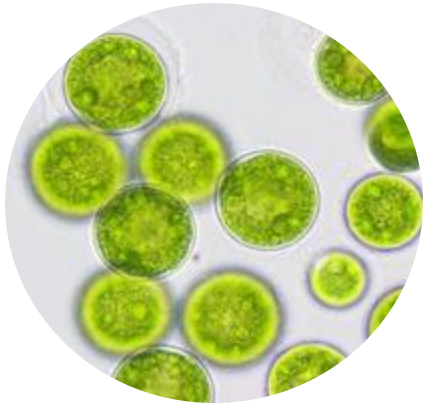
- Enzymatic functionalization & texturization
- Fermentation
- Tissue Engineering

# Fermentation includes cultivation of any microbial species for either whole-cell biomass or a higher value ingredient

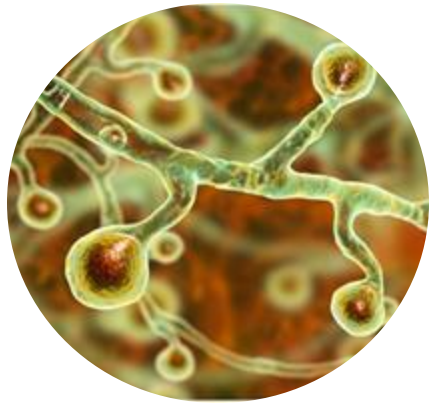
Bacteria

Yeast

Algae



Fungi



# Fermentation is expanding our ability to innovate in many categories of food

**Traditional fermentation**

- Uses **intact live microorganisms** to modulate and process plant-derived ingredients.
- Produces unique flavor and nutritional profiles, modified texture.
- Examples: Fermenting soybeans into tempeh, using lactic acid bacteria to produce dairy.

**Biomass fermentation**

- Leverages microorganism **fast growth** and **high protein content** to produce large protein quantities.
- Biomass serves as predominant or primary ingredient of a food product.
- Examples: Quorn's and Meati's filamentous fungi as base for meat analogs.

**Precision fermentation**

- Uses **microbial hosts** to produce **specific functional ingredients** that often require greater purity.
- Ingredients enable improved sensory characteristics, functional attributes.
- Examples: Perfect Day's dairy proteins; Clara Foods' egg proteins; Impossible Foods' heme; enzymes; flavoring agents; vitamins; pigments; fats.

# Precision fermentation addresses key functional & sensory challenges in plant-based products and cultivated meat

Clara Foods

Egg white proteins

Perfect Day


Milk proteins (casein, whey)

Geltor

Collagen proteins

IMPOSSIBLE™

TRITON ALGAE INNOVATIONS

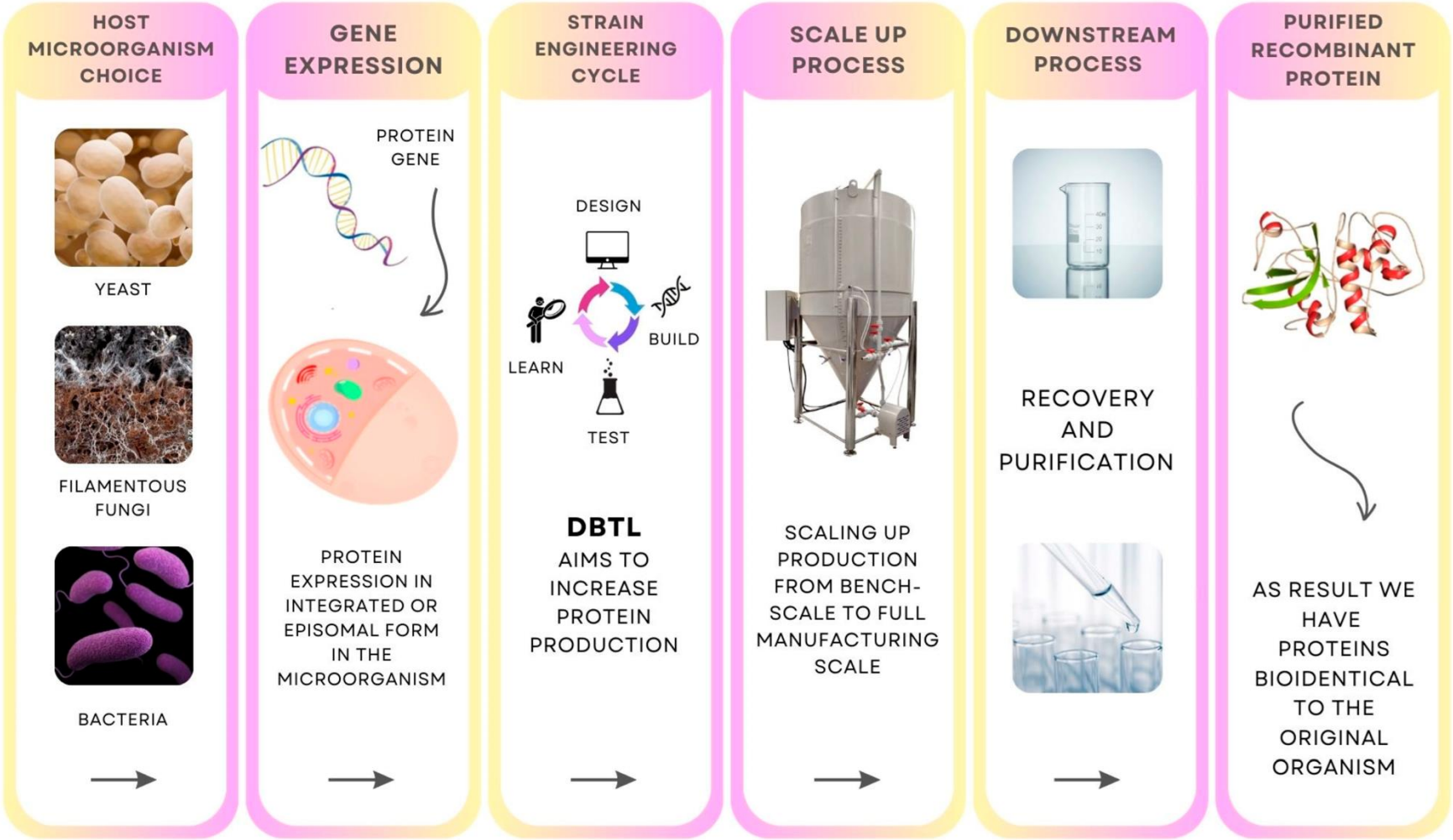
Heme proteins

RICHCORE

Growth factors

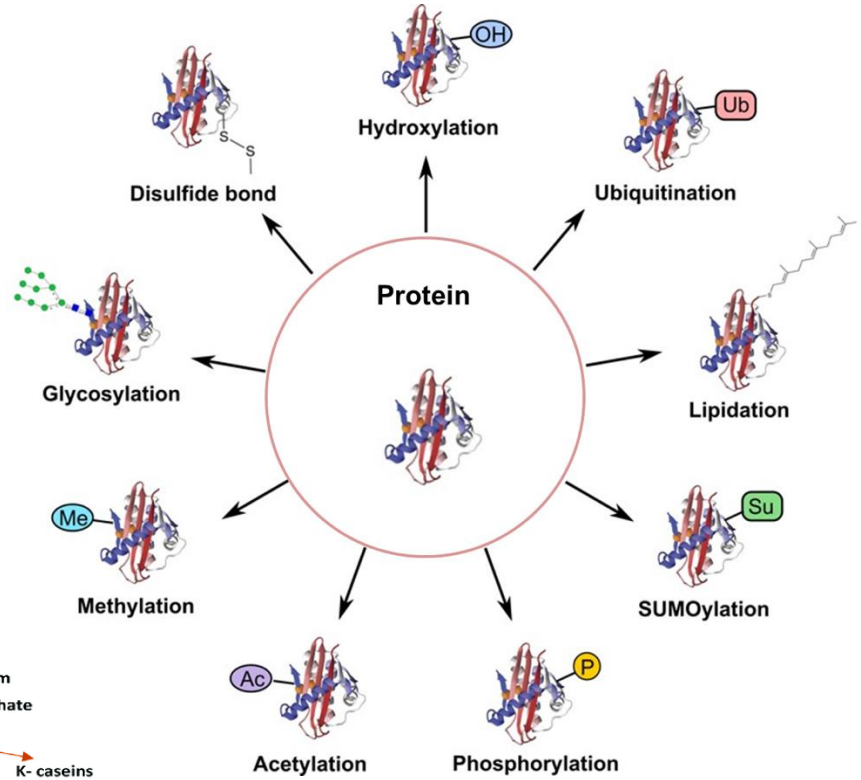
Source: Good Food Institute Fermentation 101 Lecture

# Precision fermentation allows one to *theoretically* make a bioidentical protein



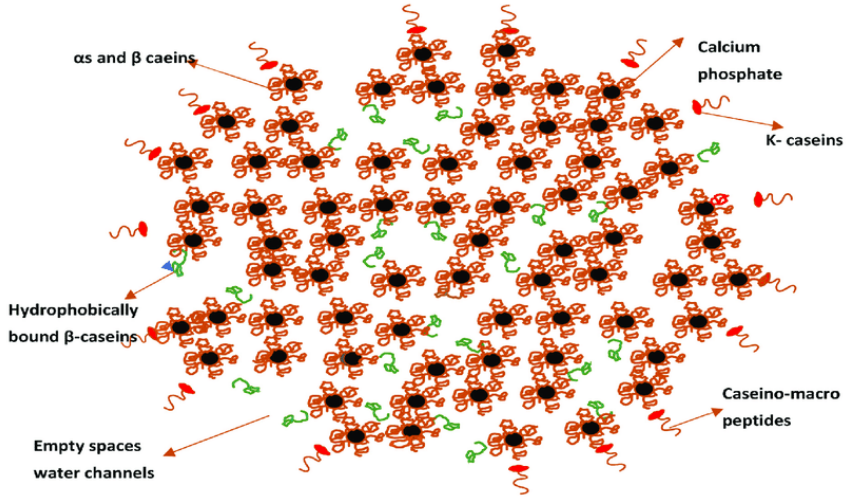
# Discussion: What are major challenges with creating structural proteins using precision fermentation?

- Microbes cannot always always add post-translational modifications
- Proper protein folding using chaperones
- Protein stability during post-processing
- Genetic stability of gene in microbe
- Yields/titers

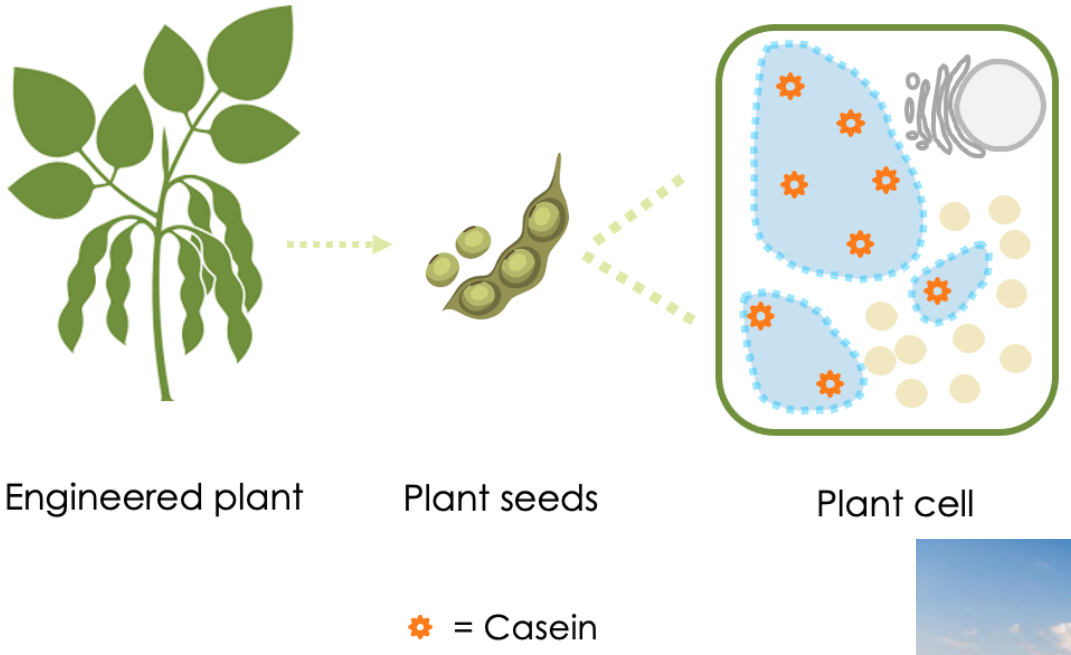



Ex: Casein post-translational modifications

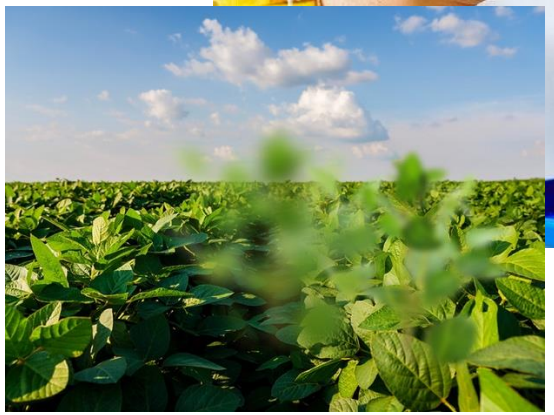
- Phosphorylation of serine and threonine
- Glycosylation of threonine
- Disulfide bond formation of cysteines
- Proteolysis (pre-degradation)
- Critical for forming casein micelles!!!



# Precision fermentation in plants may be next?







Plants can accomplish more post-translational modifications than bacteria

# Production technology landscape

EXISTING

Mixing, molding, forming

Twin screw high moisture extrusion

Low moisture extrusion

MECHANICAL INNOVATION

3D printing & electrospinning

Shear cell technology

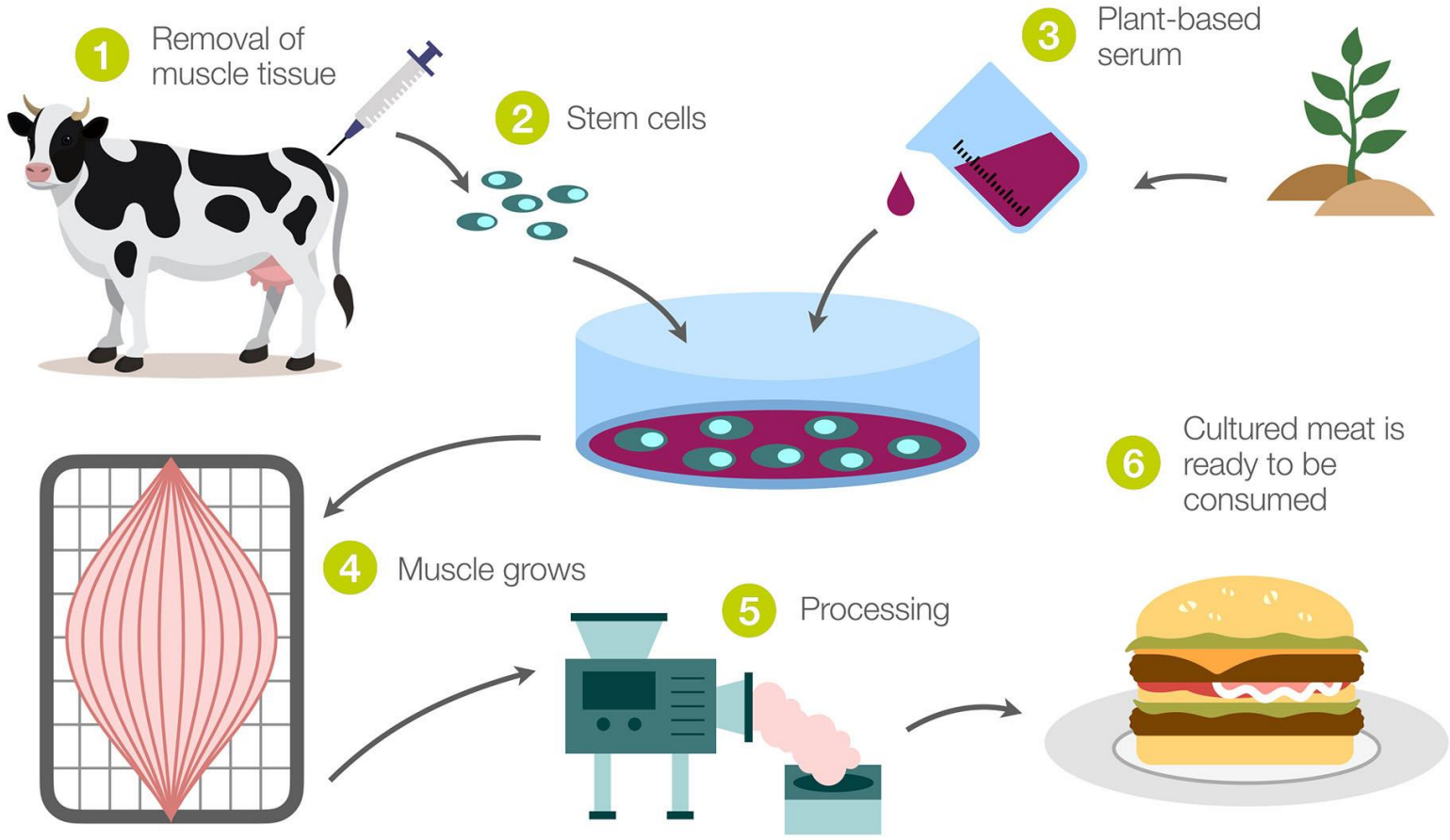
BIOLOGICAL INNOVATION

Enzymatic functionalization & texturization

Fermentation

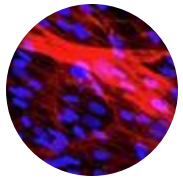
Tissue Engineering

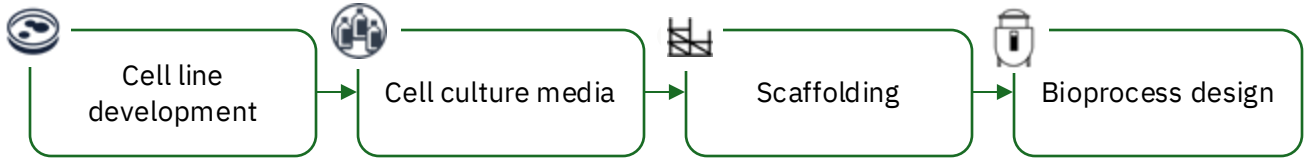
# Cultured meat involves differentiating animal stem cells into muscle cells then proliferating them



# Cultured meat involves replicating animal tissue biologically

- **Core research areas**






- **End product**

# Cultured meat is a very new field of research

**2013 Prof Mark Post**
World's First
Cultivated Burger

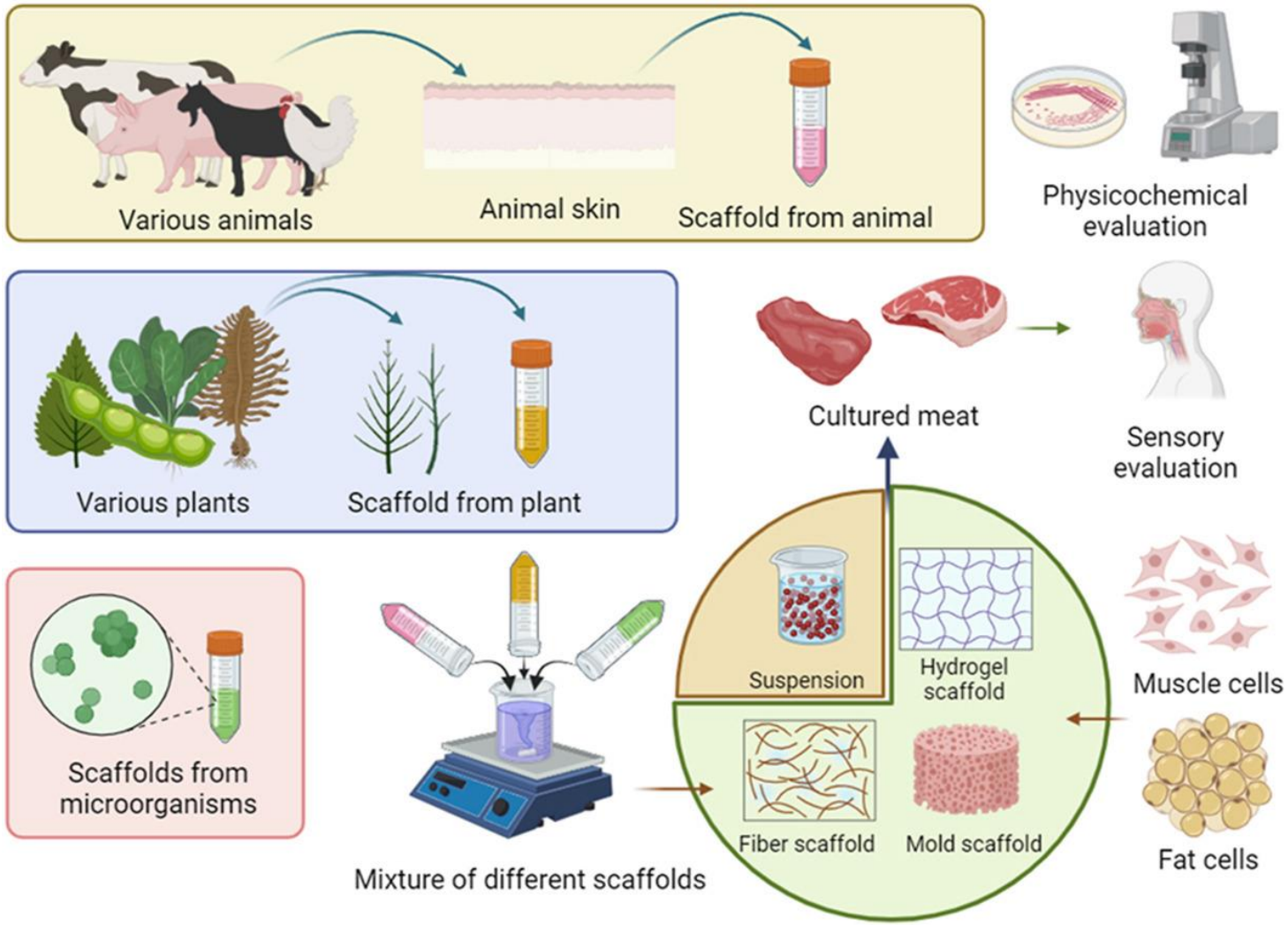
**2016 Memphis Meats**
World's First
Cultivated Meatball

**2017 Finless Foods**
World's First
Cultivated Fish

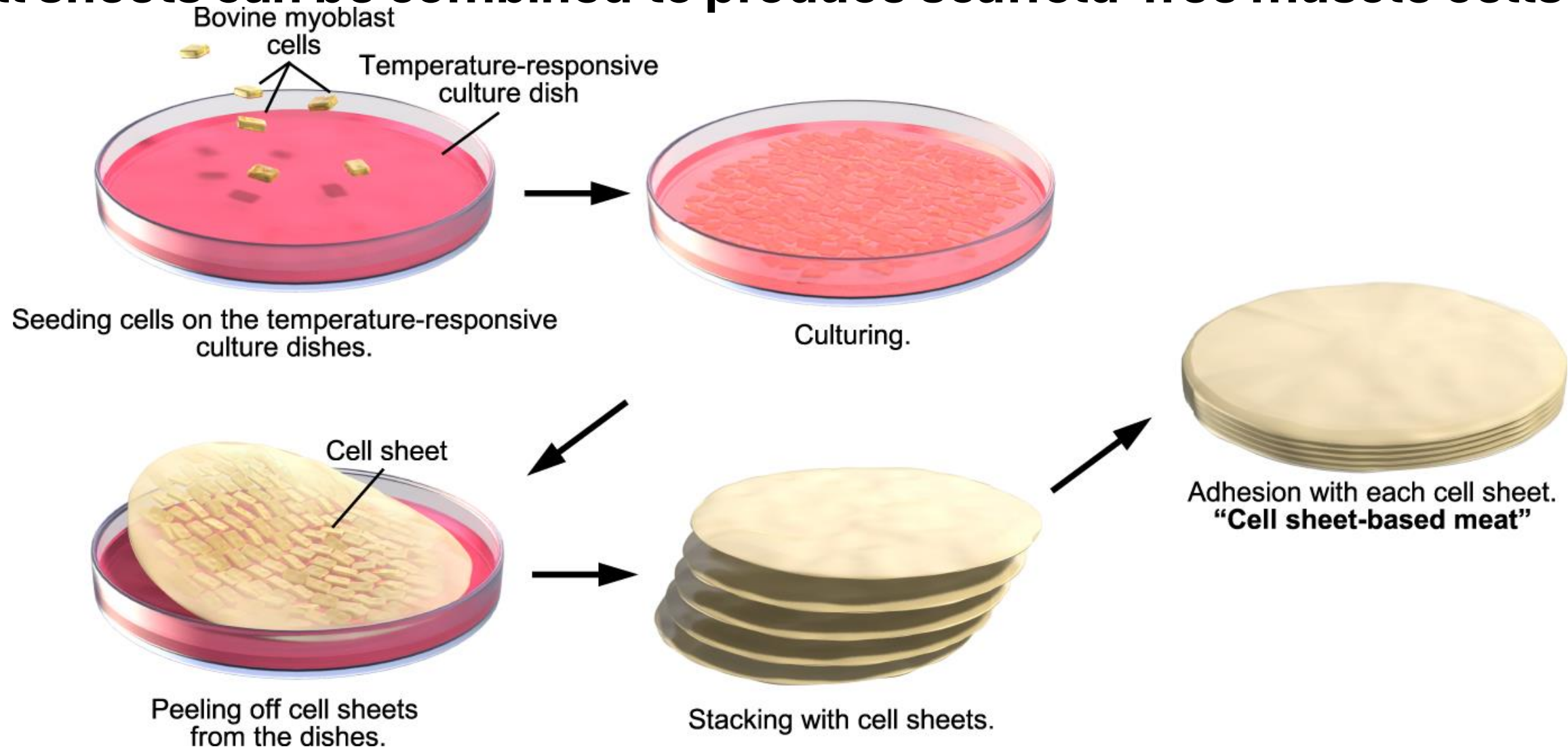
**2019 Shiok Meats**
World's First
Cultivated Shrimp
Dumpling

# Major material challenge: animal cells like to grow on scaffolds

- Scaffolds can be plant, animal, or microorganism-derived
- 2D or 3D depending on application
- Made of proteins or starches depending on cell type and source



# Cell sheets can be combined to produce scaffold-free muscle cells




Tanaka et al. Production of scaffold-free cell-based meat using cell sheet technology. *Npj Science of food* 2022.