

# Enzyme & Microbial Safety and Technology

Workshop for Feed Latina – December 14-15, 2017

DuPont Industrial Biosciences Product Stewardship & Regulatory

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# Agenda

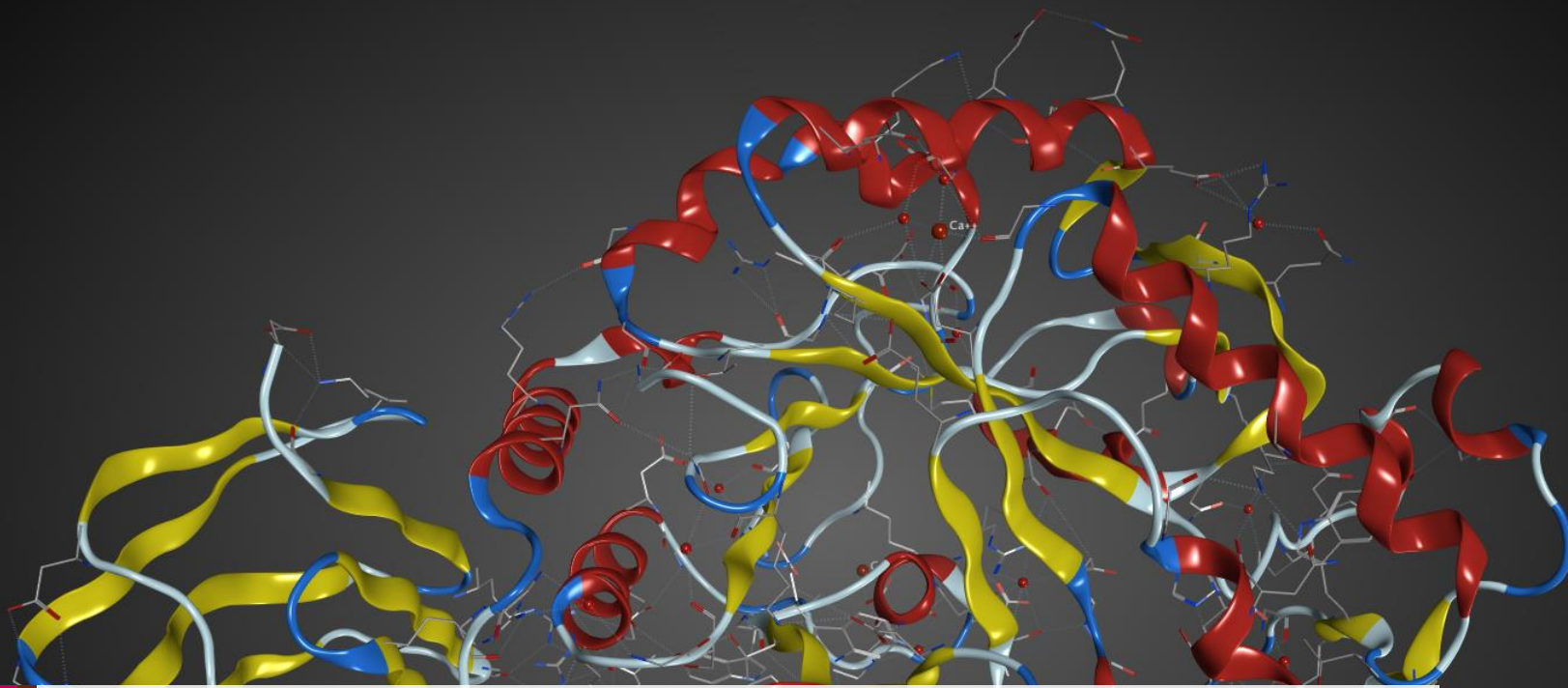
Timing	Agenda	Topics
Thursday	Enzyme & Microbial Safety	<ol style="list-style-type: none"><li>1. What are enzymes (basics)</li><li>2. Enzyme &amp; DFM uses</li><li>3. Safety evaluation</li></ol>
Friday	Enzyme Technology	<ol style="list-style-type: none"><li>1. What are enzymes (more detail)</li><li>2. How are enzymes developed and manufactured<ul style="list-style-type: none"><li>- Production organisms</li><li>- Fermentation</li><li>- Downstream processing</li></ul></li><li>3. 'Enzyme' vs 'enzyme preparation'</li><li>4. Storage and handling</li></ol>



# Enzyme Safety

- What are Enzymes ?
- Enzyme Uses
- Enzyme Safety Assessment

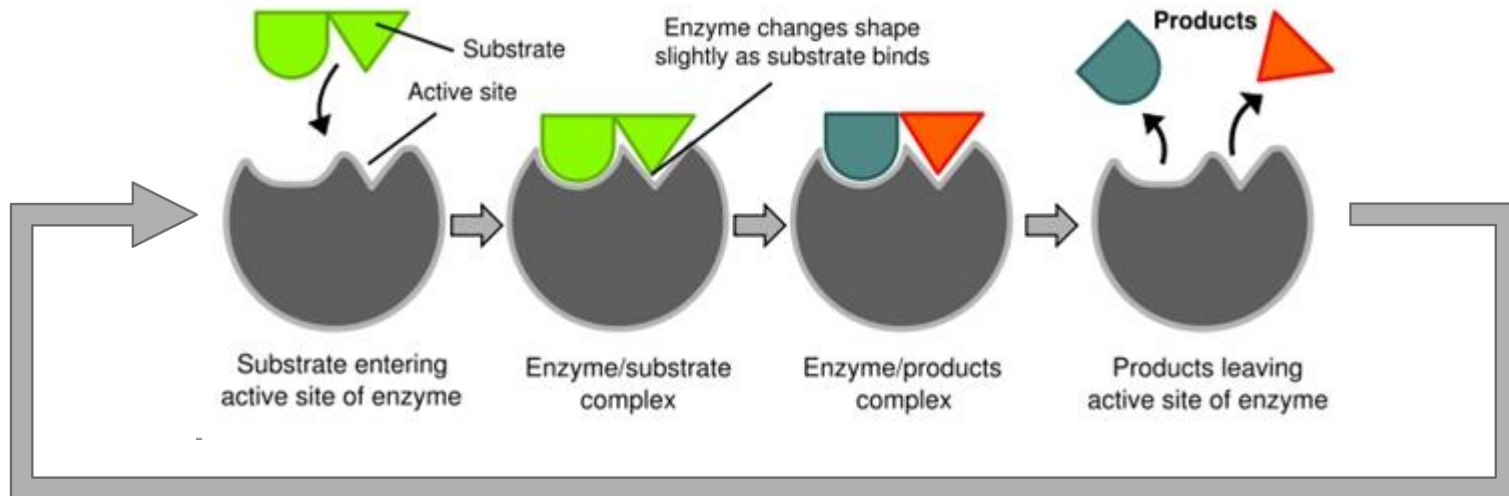




**What are Enzymes ?**

# Enzymes Are Natural Catalysts

- They speed up chemical reactions, lowering the energetic threshold
- They are specific (substrate + reaction)
- Required in very small amounts as they are not consumed during the reaction



## Classification according to reaction catalyzed by the enzyme:

- International Union of Biochemistry and Molecular Biology
- <http://www.chem.qmul.ac.uk/iubmb/enzyme/>

## Enzymes Work by Targeting Specific Substrates

Substrate	Effect of substrate	Enzyme
Soluble viscous NSPs (e.g arabinoxylans)	<ul style="list-style-type: none"> <li>↑ viscosity and digesta retention time</li> <li>↓ nutrient absorption</li> <li>↑ proliferation of intestinal microflora</li> </ul>	Xylanase
Insoluble, non-viscous NSPs	<ul style="list-style-type: none"> <li>↓ accessibility of nutrients by physical entrapment</li> </ul>	Xylanase
Starch	<ul style="list-style-type: none"> <li>Metabolisable energy</li> <li>↑ substrate for gut microflora</li> </ul>	Amylase
Protein	<ul style="list-style-type: none"> <li>Metabolisable energy and AA</li> <li>↑ substrate for gut microflora (neg)</li> </ul>	Protease
Lipid	<ul style="list-style-type: none"> <li>↑ emulsification, digestibility of lipids</li> </ul>	Lipase
Raffinose and stachyose	<ul style="list-style-type: none"> <li>Undigestible by animal enzymes</li> </ul>	α-galactosidase
Beta glucan	<ul style="list-style-type: none"> <li>↑ viscosity and digesta retention time</li> </ul>	β-glucanase
Phytate	<ul style="list-style-type: none"> <li>Binds minerals, protein and starch</li> </ul>	Phytase

# Enzyme definitions

## Enzyme preparation

= product that is actually sold

## Enzyme concentrate

= “feed enzyme”

Usually the subject of approval

## Enzyme protein

Pure substance

Laboratory

White crystals

Fermentation extract

Enzyme factory

Brown liquid

Formulation

Food manufacturers

Liquid or granulate

# How are enzymes used?

## - additives vs processing

### Additives



**Animal Nutrition**



**Home Care**  
- Detergent  
enzymes



**Grain Processing:**  
- Syrups  
- Fermentation

### Processing



**Food Processing:**  
Baking, Dairy &  
Brewing



## Xylanase, Amylase, Protease: Mode of action summary

Xylanase  
fibre degrading  
enzymes

**X**

- open up cell walls
- reduce digesta viscosity
- improve digestion
- increase feed intake
- reduce microbial fermentation
- reduce endogenous losses

Amylases

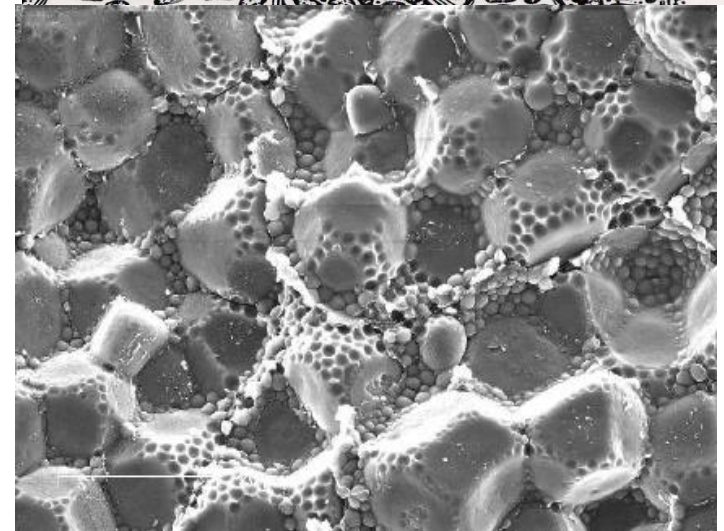
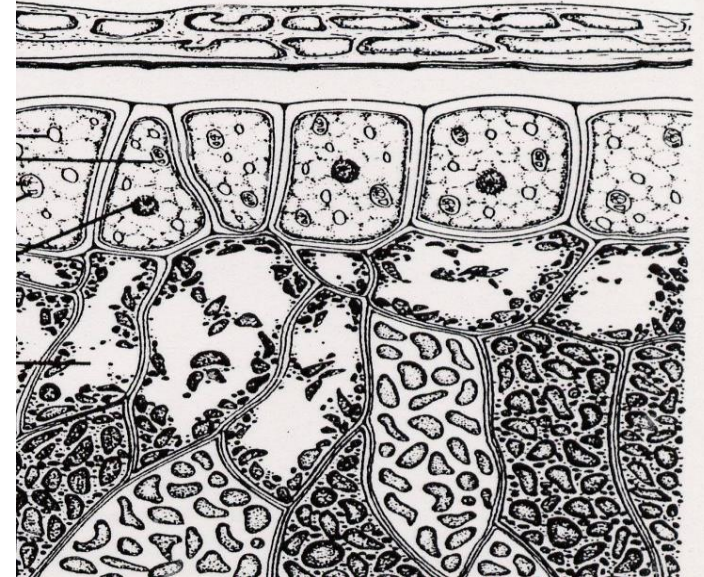
**A**

- degrade starch
- improve energy digestion

Proteases

**P**

- Protein encapsulated starch
- Target storage proteins
- Improve digestion
- Degrade antinutrients in Soy (trypsin inhibitor)



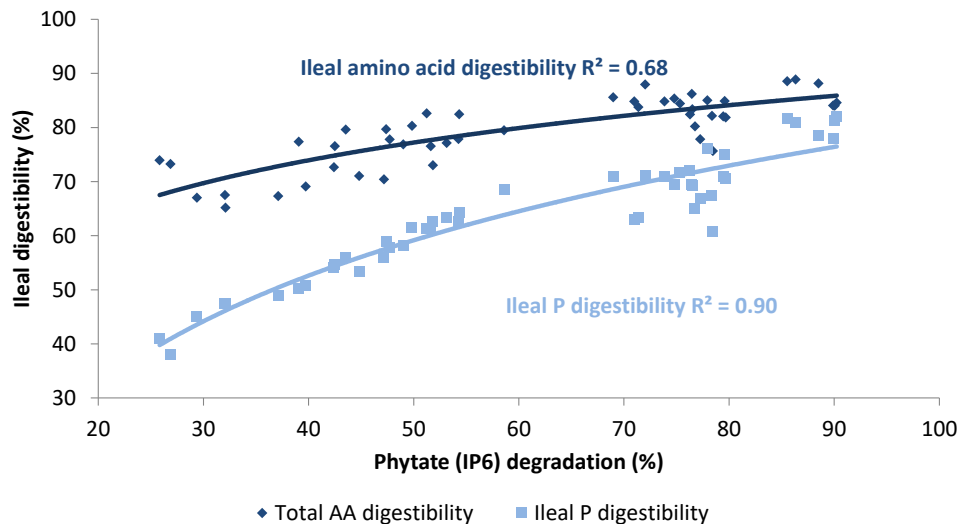
# Environment benefits

## Amylase, Xylanase, Protease

- Improves amino acid/crude protein digestibility = reduced nitrogen excretion

## Phytase

- Improves phosphorus digestibility = reduced phosphorus excretion



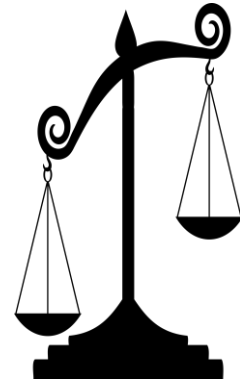
## General Enzyme Safety (Animals)

### Animal Feed Safety

- Proteins are an essential part of the diet
- Enzymes are inherently present in grains and beans and are thus consumed by animals in their active state or as inactive proteins (in processed feed) every day.
- Enzymes are broken down into simpler components such as peptides and amino-acids, which are then entirely metabolized by the body.

## General Enzyme Safety (Weight-of-Evidence)

- Enzymes have low acute toxicity
  - LD50's > 5000 mg/kg
  - Enzymes lack sub-chronic oral toxicity:
  - The enzyme industry has conducted more than 200 90-day oral gavage or feeding studies with numerous enzymes derived from multiple strains with no adverse findings observed.
- Enzymes lack genotoxicity:
  - The enzyme industry has conducted more than 217 and 226 mutagenicity studies, respectively on bacterial (i.e., Ames) and mammalian cells (e.g., chromosomal aberration studies) on a wide variety of enzymes from multiple strains- all testing was negative
- Enzymes are used at very low levels (e.g., ppm)
  - Any contaminants, if present, would be at '*de minimis*' levels.



# General Enzyme Safety (Occupational Handling)

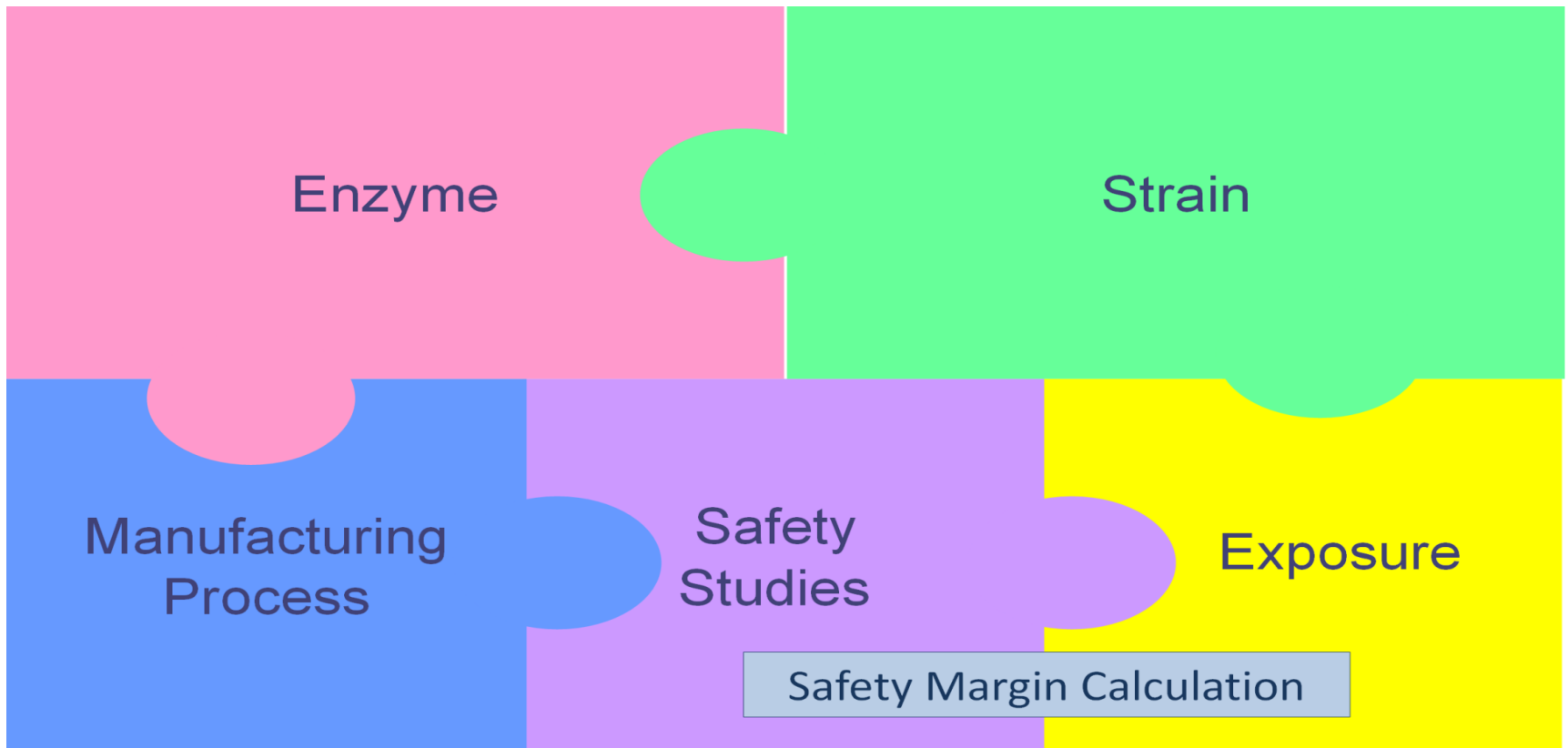
## Industry Handling safety

- Enzymes are not skin irritants or sensitizers or eye irritants
  - Exception: proteases can irritate skin and mucous membranes if at high concentrations
- As with any protein, enzymes, when inhaled have the potential for sensitization and to elicit a respiratory allergic response





## Safety Evaluation of Feed Enzymes: 5 Essential Elements:



# Enzyme

## Characterization

- IUBMB and CAS classification
- Source
- Functionality
  - Specific reaction
- Properties
  - MW, structure, protein sequence

## History of Safe Use (for animals)

- Alternatively, assessment of the protein sequence for similarity to known protein toxins and allergens
  - Considers source, type and history of safe use of protein
  - Sequence evaluation approach based on published methods (Ladics et al., 2011)

## 1. Strain Characterization

- Safety of production strain is key component to safety evaluation
- Non-toxigenic
- Concept → If the production organism is safe then the ingredient produced is safe.

## 2. Genetic engineering of host

- Non-toxigenic
- Do not encode or express any harmful substances

## 3. Introduced DNA

- Well-characterized
- Use common techniques
- Description of source for expressed gene
- Well-known plasmids and selectable markers

(no transferable antibiotic resistance markers of clinical relevance)

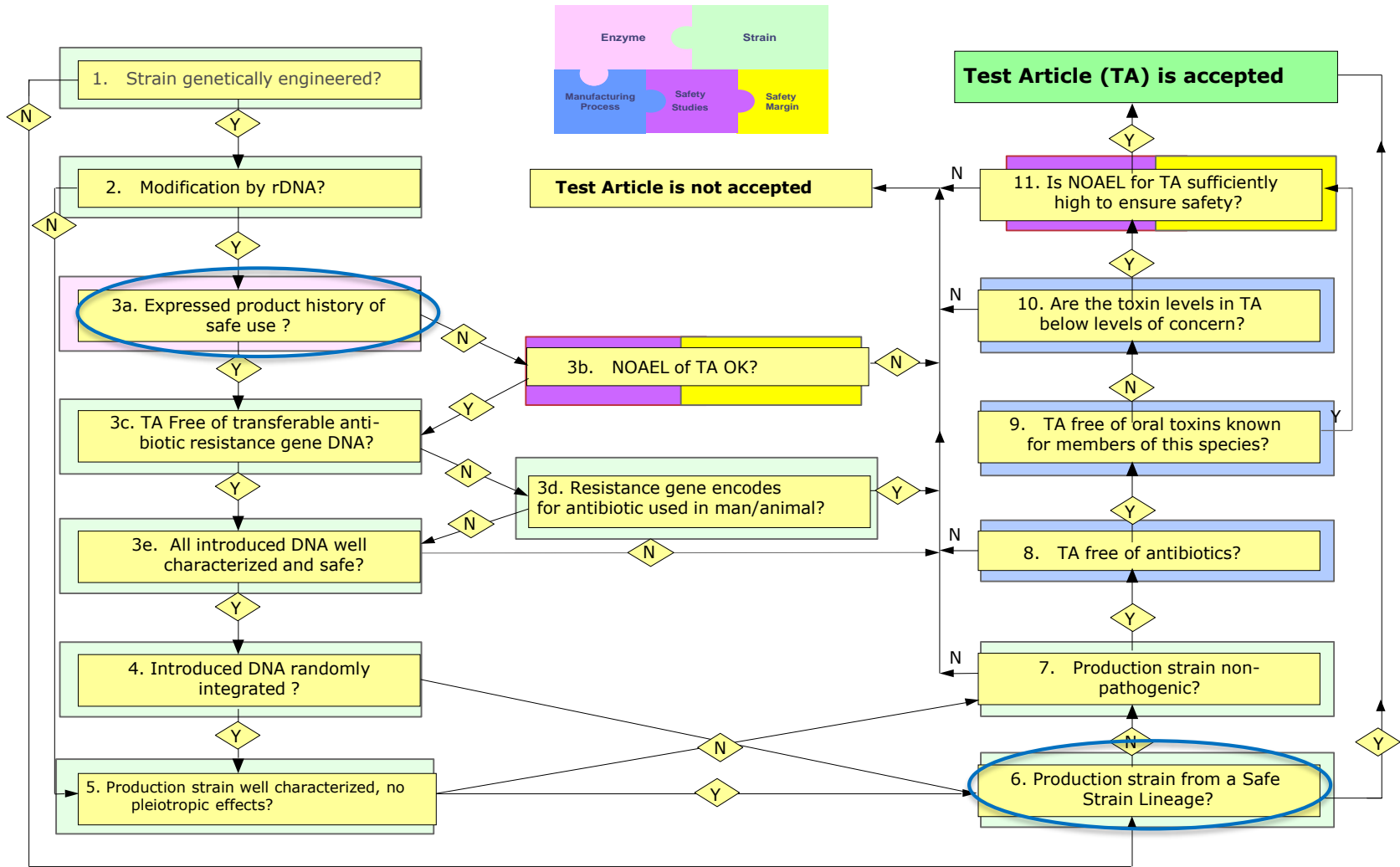


Strain

## 4. Demonstration of Safe Strain Lineage

- History of safe use in food and for production of enzymes
- Safety demonstrated by repeated tox studies and analysis using decision tree guidelines

# Pariza & Cook (2010) Decision Tree

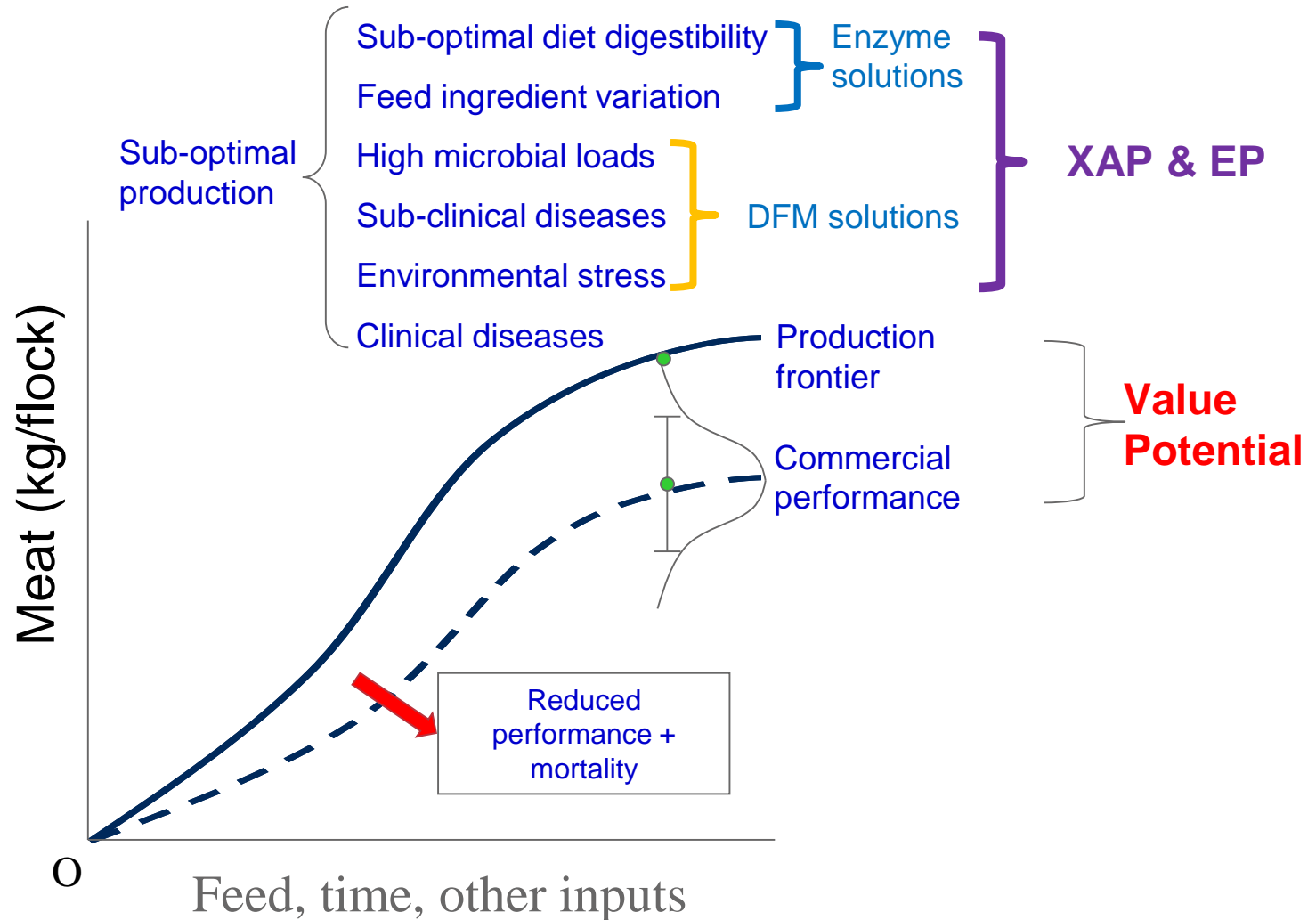


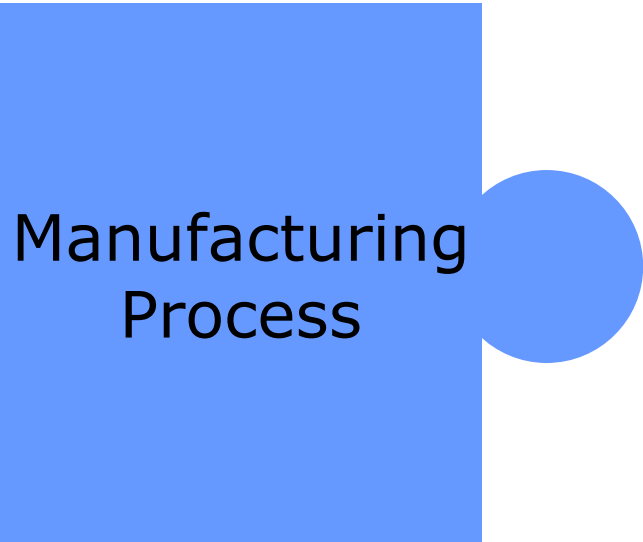


## DFMs

- Safe Species
  - » Listed as suitable as live microbes in AAFCO Official Publication
  - » Qualified Presumed Safe (QPS) in Europe
    - » *Bacillus amyloliquefaciens*, *B. licheniformis*, *B. subtilis* a.o.
      - » Predicated upon the strains not producing toxins (similar to enzyme production strain requirement)
    - » Lactic acid bacteria
- Usually marketed as spores
  - » Stable product form that can survive pelletizing
- Combination products with enzymes to optimize gut function

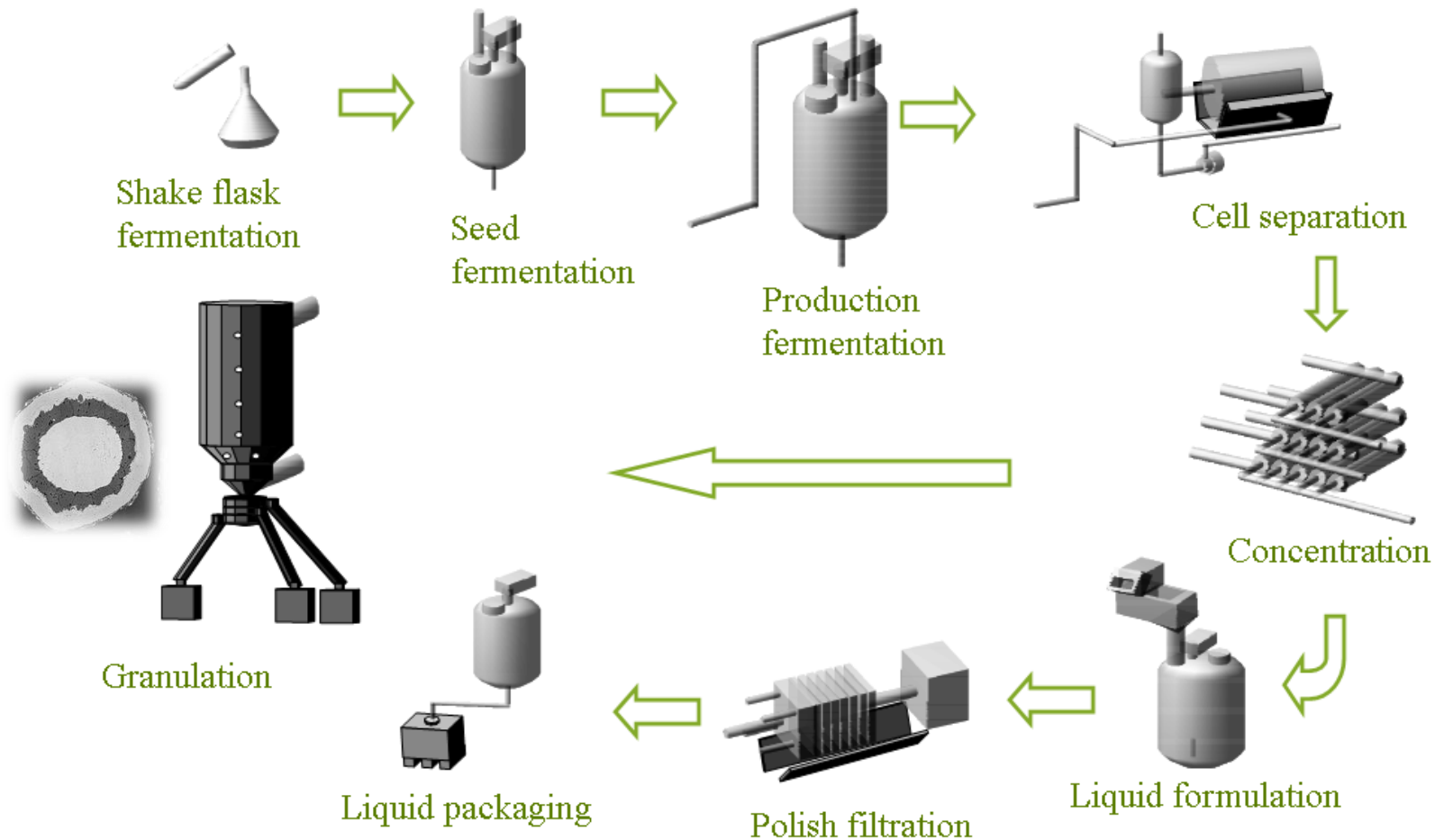
Only an optimised combination of Enzyme and Probiotic can help you realise the full potential of the bird



A large blue graphic element consisting of a square on the left and a circle on the right, both in a solid blue color. The text "Manufacturing Process" is centered within the square.

# Manufacturing Process

# Manufacture of Enzymes



# Manufacturing Process

Produced in accordance with Good Manufacturing Practices

- Pure culture fermentation
- Safe raw materials and processing aids

Quality Management system (e.g., ISO 9001)

Controlled Manufacturing Process

- Contained and controlled fermentation
- Recovery (e.g., cell separation, concentration)
- Formulation
- Quality checks for contamination
- Product Specifications
  - microbial and metal contaminants
  - as provided by JECFA and Food Chemical Codex for food enzymes



## Safety Studies

### Testing done depending on decision tree guidance

Characterization of test article

- Representative of the commercial products

### For animals:

- Sub-chronic oral toxicity study
  - 90-day repeated oral dose study with rodents
    - To establish No Observed Adverse Effect Level (NOAEL)
- Genotoxicity tests as needed for regulatory requirement
  - Ames Assay
  - Chromosomal Aberration
- Alternatively: tolerance trial in most sensitive target specie

### For exposure to humans (via handling of enzymes):

- Acute inhalation
- Skin irritation
- Eye irritation



## Enzyme Exposure

### ■ Dosage

- ❑ Use rate in application – generally very low (<0.1%)
- ❑ Quantified as Total Organic Solids (TOS) from the fermentation
  - » (enzyme protein, peptides, amino acids, soluble sugars, metabolites)

### ■ Consumption

- ❑ Food intake per kg/bw

### ■ Exposure

- ❑  $\text{Exposure} = \text{Dosage} \times \text{Intake}$

### Safety Margin

- Dose level with No Adverse Effect (NOAEL) / Exposure
  - ❑ Conservative calculation
    - » Assumes enzyme used in all of the particular food
    - » Assumes all of the enzyme stays in the food

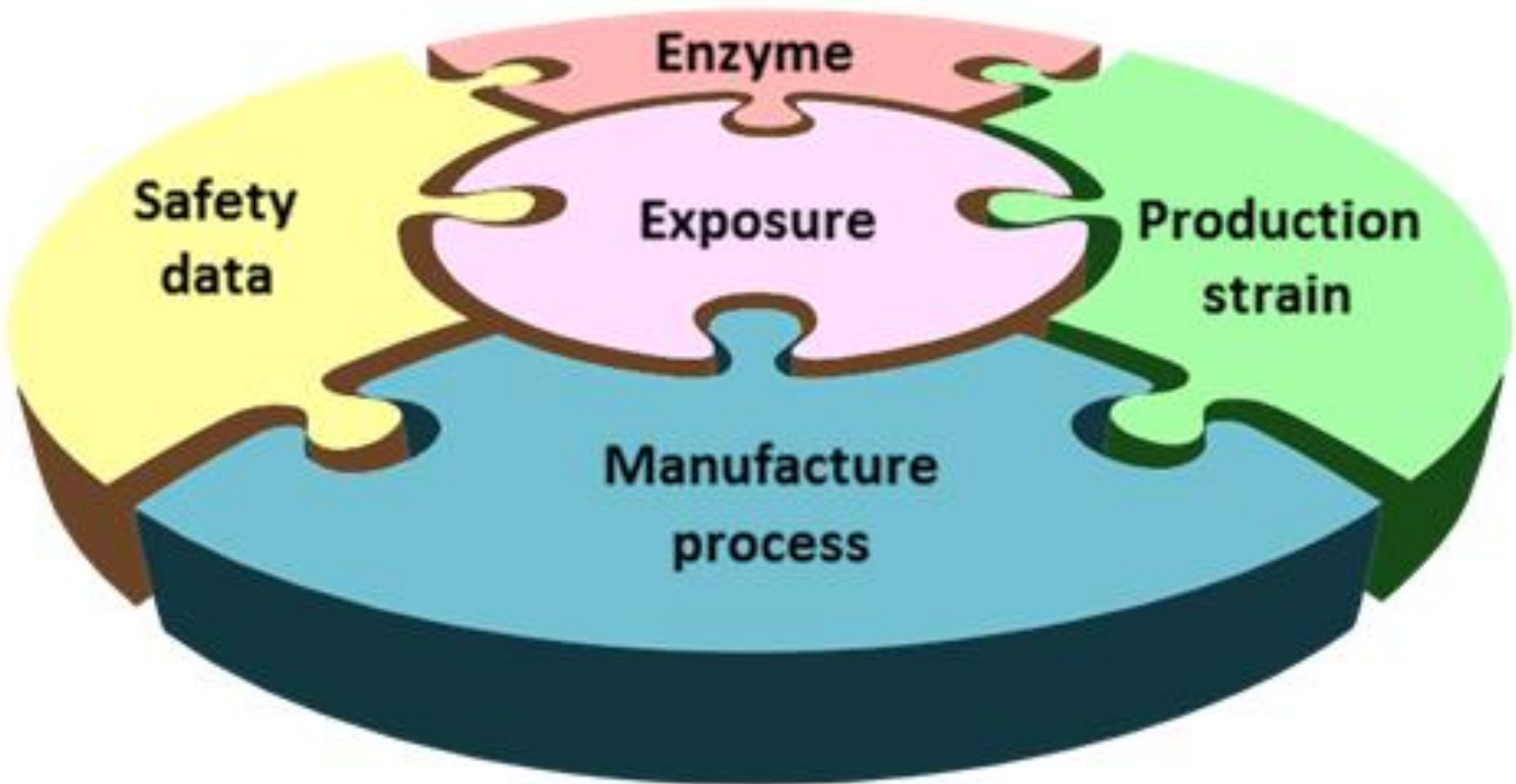
Exposure &  
Safety Margin

## Feed Enzyme Regulation- United States

- Feed enzymes, like most ingredient that become part of the animal food, are considered food additives unless they are Generally Recognized As Safe (GRAS).
  - » Need to be approved through Food Additive Petition (FAP) unless GRAS
- **Dupont IB enzymes all being determined to be GRAS**
  - » Process:
    - » Self-determination by qualified individuals
    - » Pivotal information is published
    - » Optional concurrence opinion by expert(s)
    - » Optional GRAS Notice filing with FDA
      - » To support US customer requirements
      - » To maximize transparency, e.g., via AAFCO book listing
      - » To support international acceptance (e.g., Australia, Malaysia)

## Feed Enzyme Regulation- GRAS

- GRAS is the US exemption to formal approval as food additive
- Enzymes are uniquely suited to the GRAS process
  - » Long History of Safe Use
  - » Well-established and generally recognized safety evaluation procedures (puzzle pieces and decision tree)
  - » Use of production organisms that belong to Safe Strain Lineage
  - » FDA familiar and comfortable with enzyme technology & decision tree
  - » Fit of enzymes to GRAS process discussed in publications:
    - » <http://online.liebertpub.com/doi/pdf/10.1089/ind.2016.0011>
    - » <http://www.enzymeassociation.org/wp-content/uploads/2017/08/ETA-Response-GRAS-from-the-ground-up-final.pdf>
    - » <http://online.liebertpub.com/doi/pdf/10.1089/ind.2017.29098.vjs>



**Thank You!**