

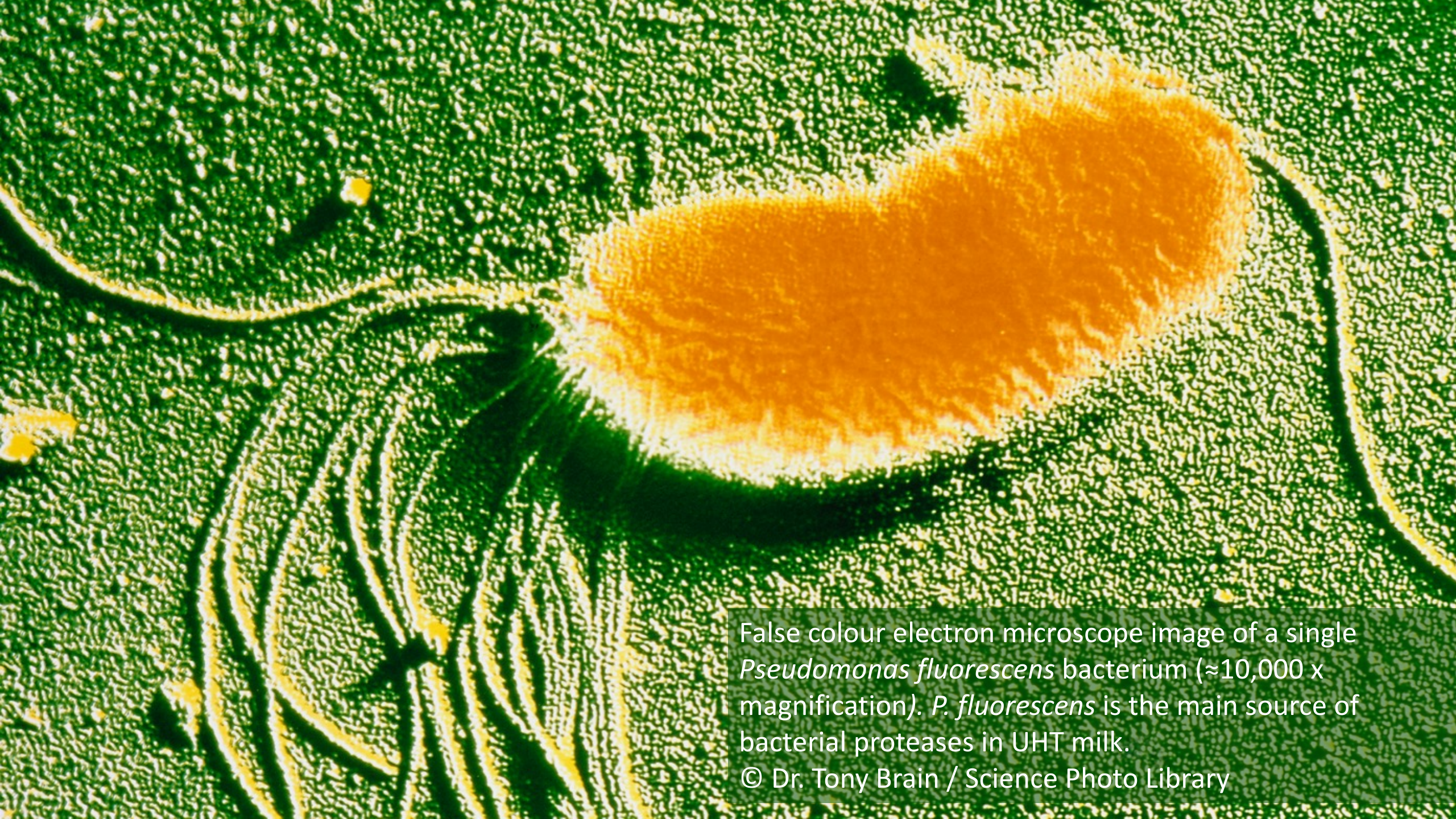
# Shelf-life failures of UHT milk and how to prevent them

Stephen Trowell, PhD



# UHT Shelf-Life and Heat Stable Proteases

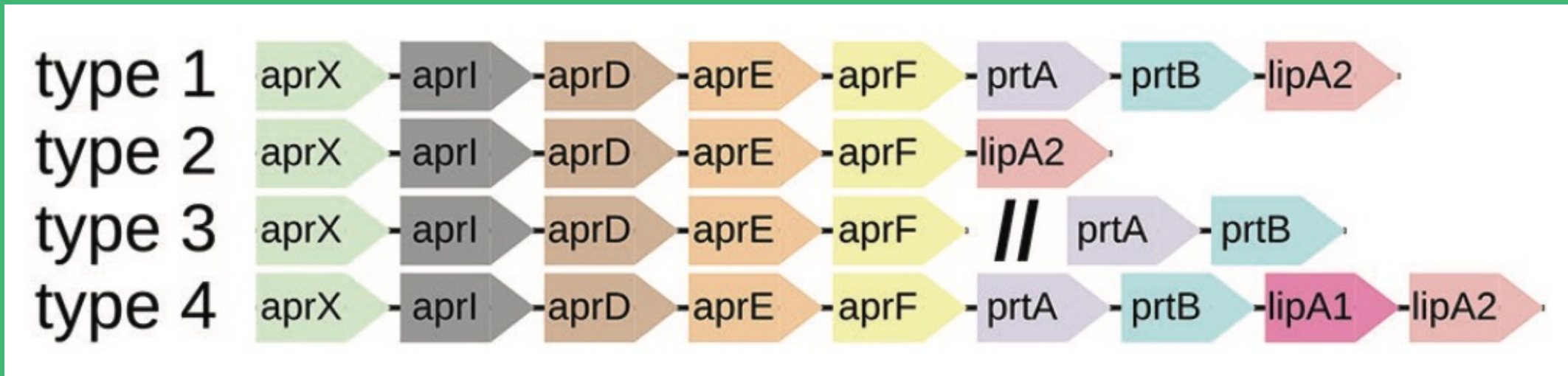
- Catastrophic failure is rare – but it happens
- Shelf-life limitation from 12 months down to 9, 6 or even 3 months
- AprX from Pseudomonas bacteria – the most common problem
- Plasmin/plasminogen from the cow – less commonly a problem
- Subtilase from aerobic bacilli – can be an issue in reconstituted powder



False colour electron microscope image of a single *Pseudomonas fluorescens* bacterium ( $\approx 10,000 \times$  magnification). *P. fluorescens* is the main source of bacterial proteases in UHT milk.

© Dr. Tony Brain / Science Photo Library

# Genetics of AprX Expression in *Pseudomonas* spp.

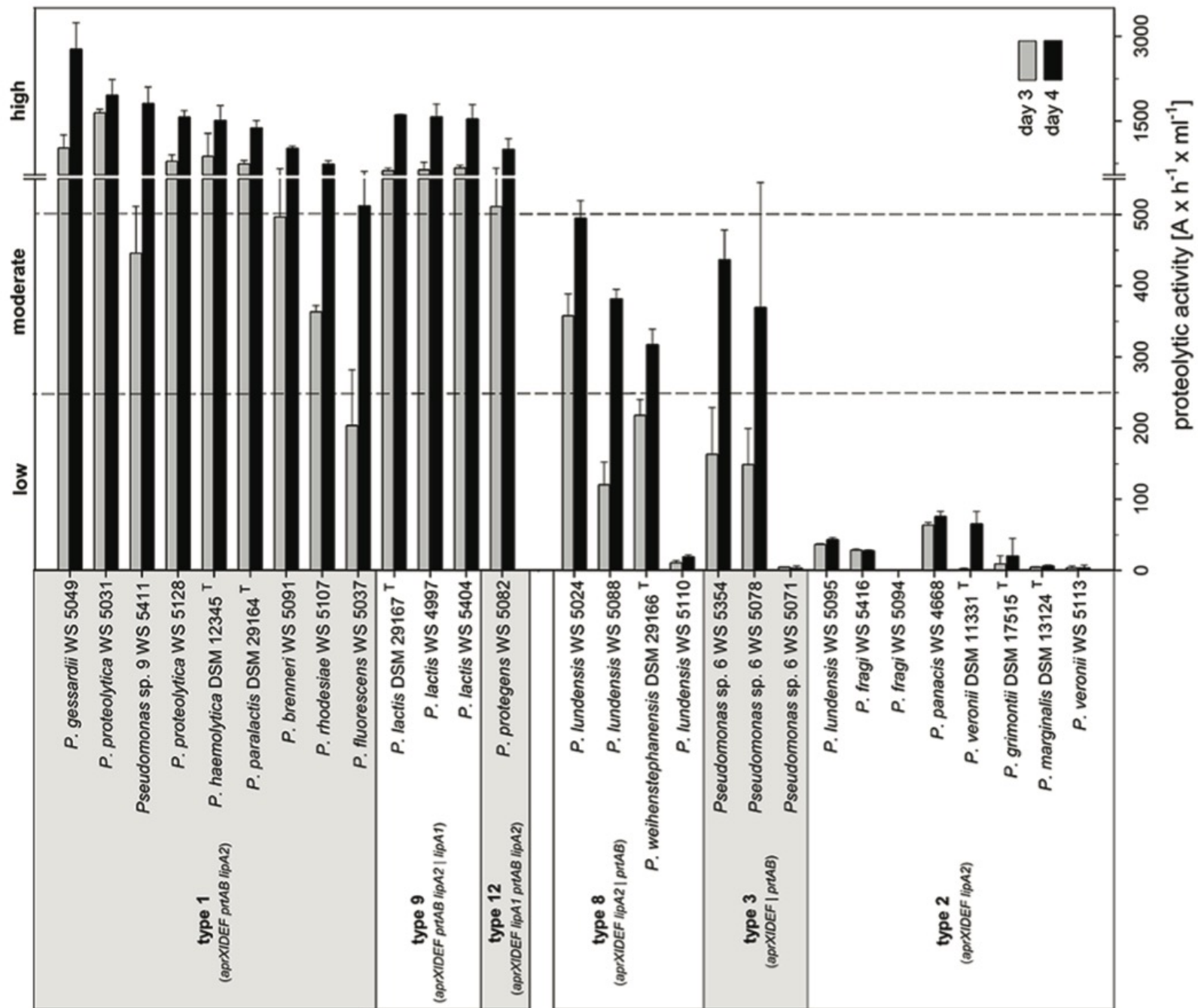


## Genetic Organization of the *aprX-lipA2* Operon Affects the Proteolytic Potential of *Pseudomonas* Species in Milk

Christopher Maier<sup>1†</sup>, Christopher Huptas<sup>2†</sup>, Mario von Neubeck<sup>1‡</sup>, Siegfried Scherer<sup>1,2</sup>, Mareike Wenning<sup>3</sup> and Genia Lücking<sup>1\*</sup>

 **frontiers**  
in Microbiology

ORIGINAL RESEARCH  
published: 10 June 2020  
doi: 10.3389/fmicb.2020.01190



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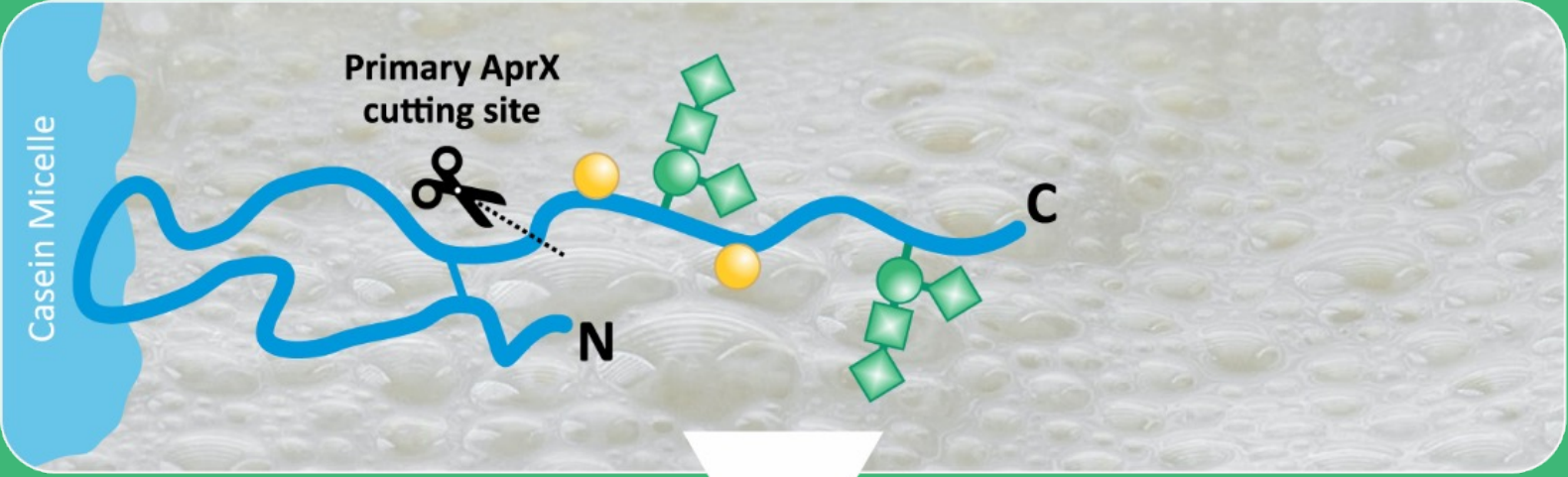
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Take home message:

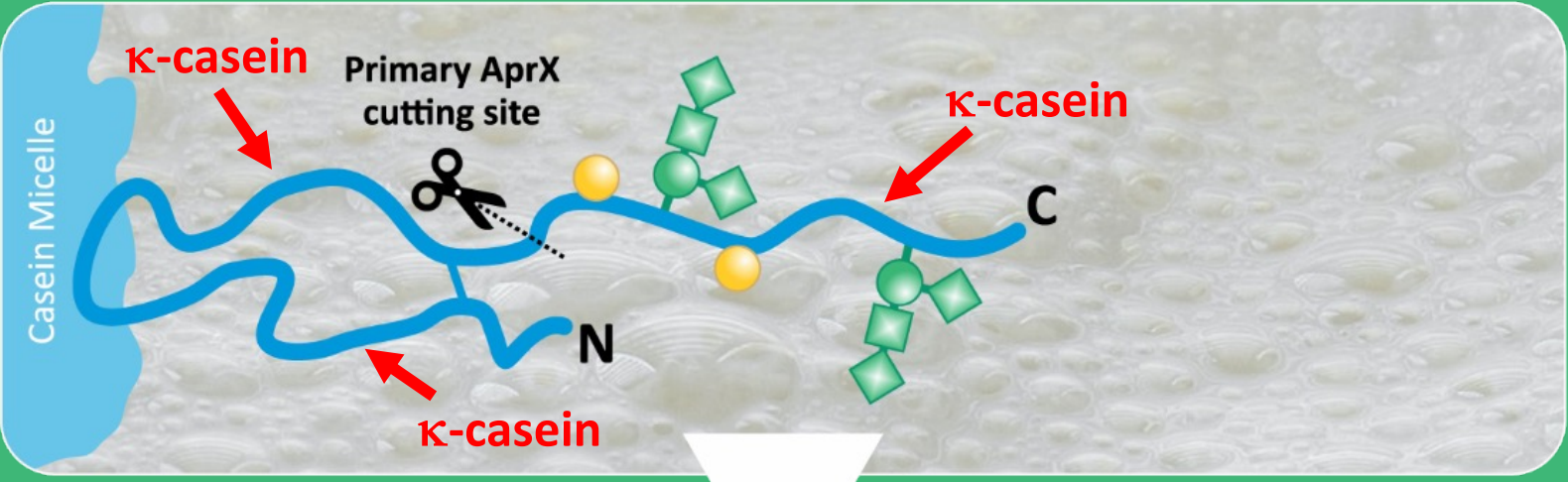
Pseudomonad colony counts are not directly related to AprX activity

# How AprX Damages Milk

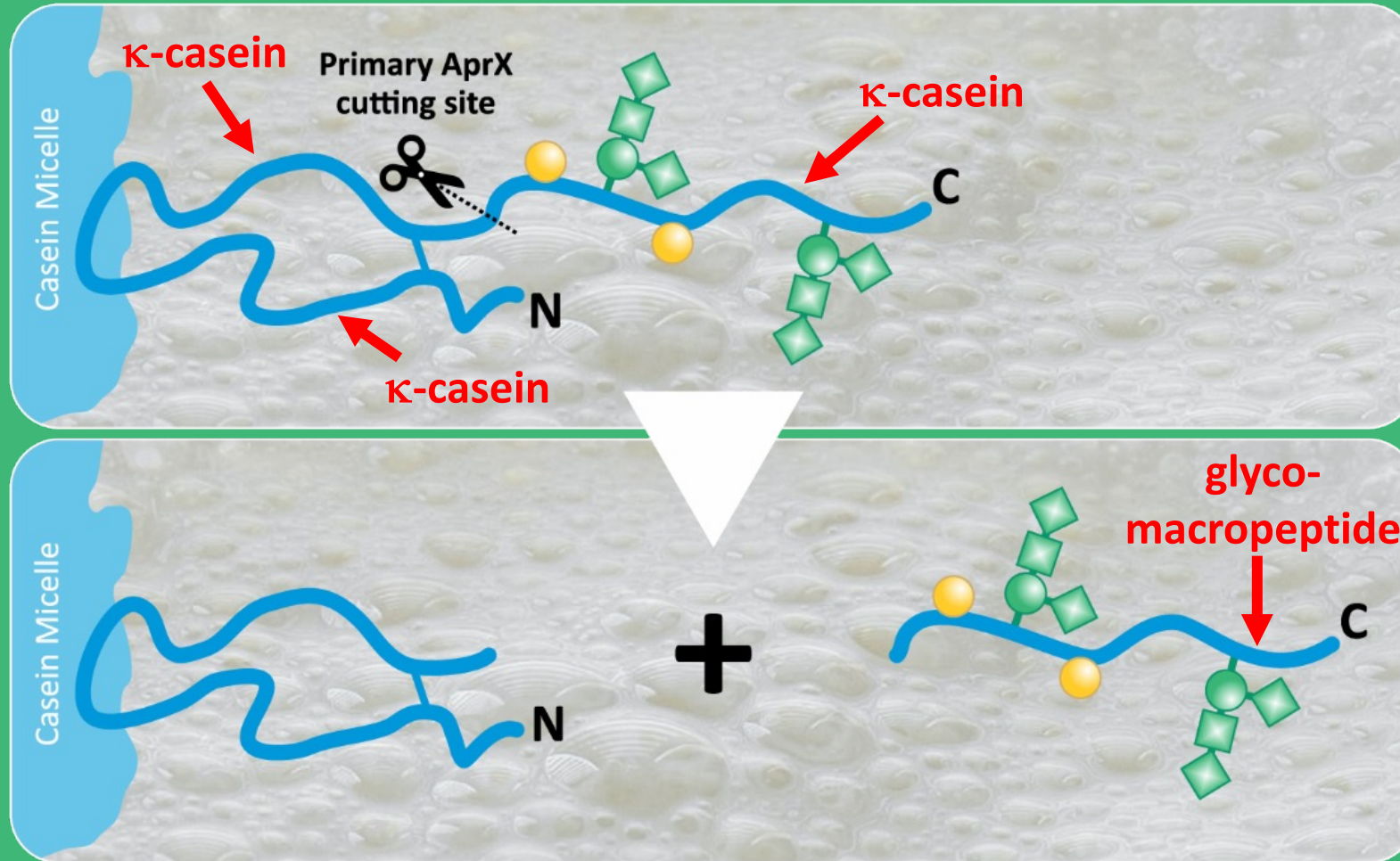




# How AprX Damages Milk



# How AprX Damages Milk

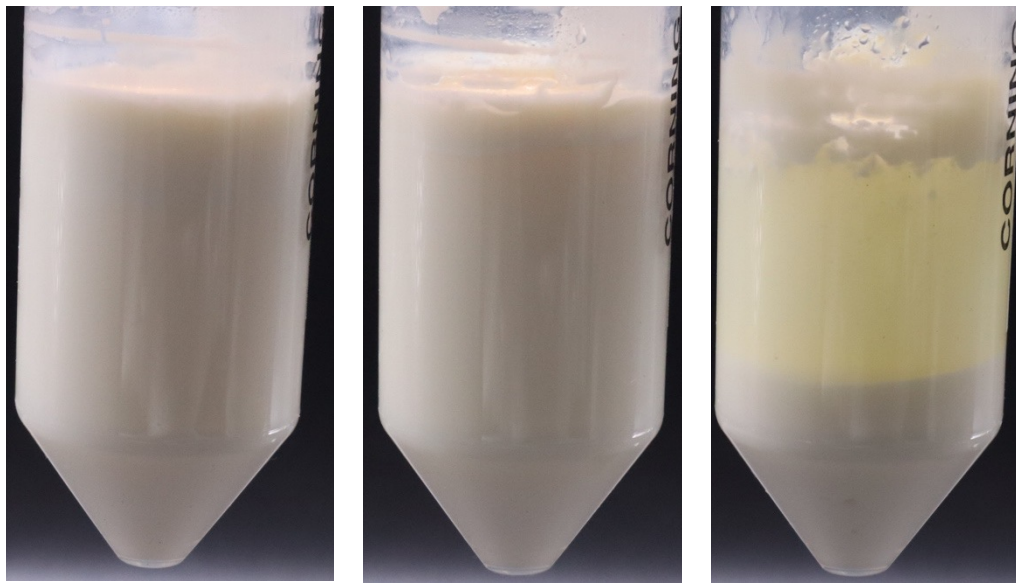




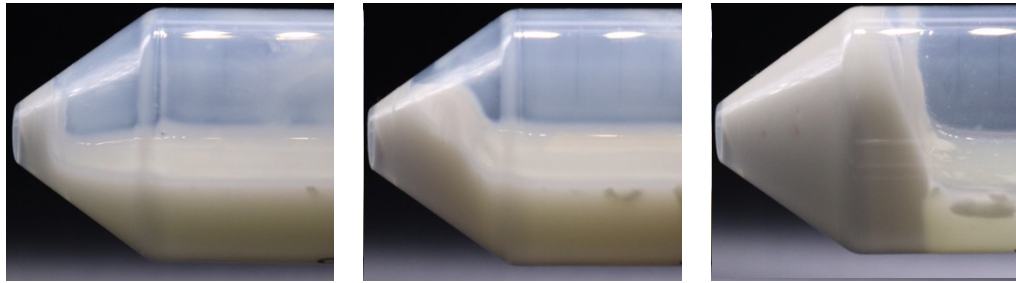
4 °C



Up to 1 year  
Any level of  
AprX activity



← 22 °C →



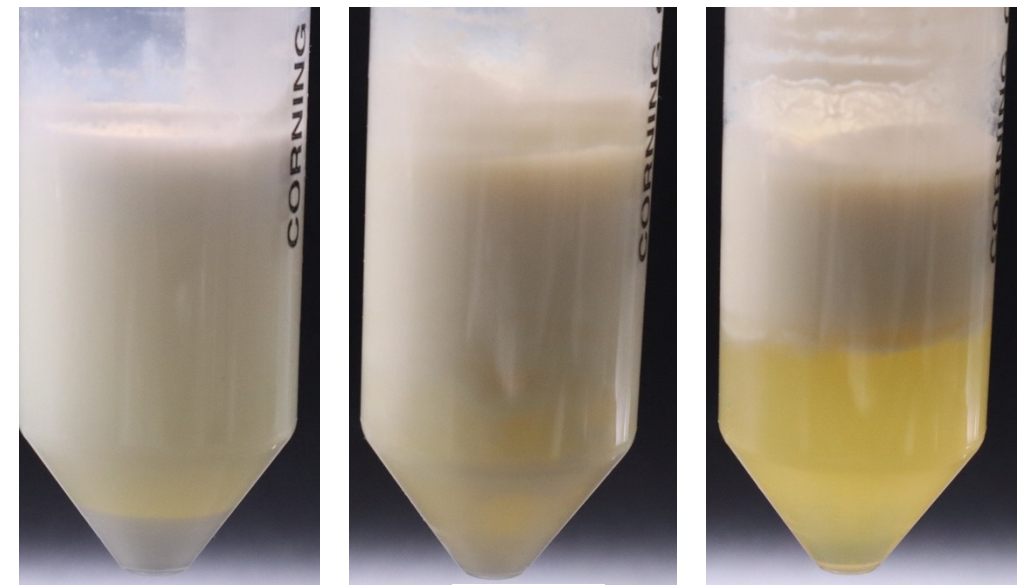
10 days

2 weeks

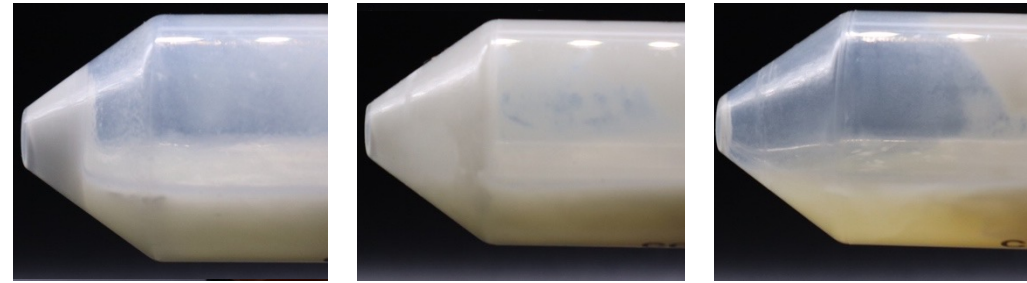
14 weeks

5  $\mu$ U/mL AprX activity

**$\kappa$ -casein selectively & progressively targeted**



← 22 °C →

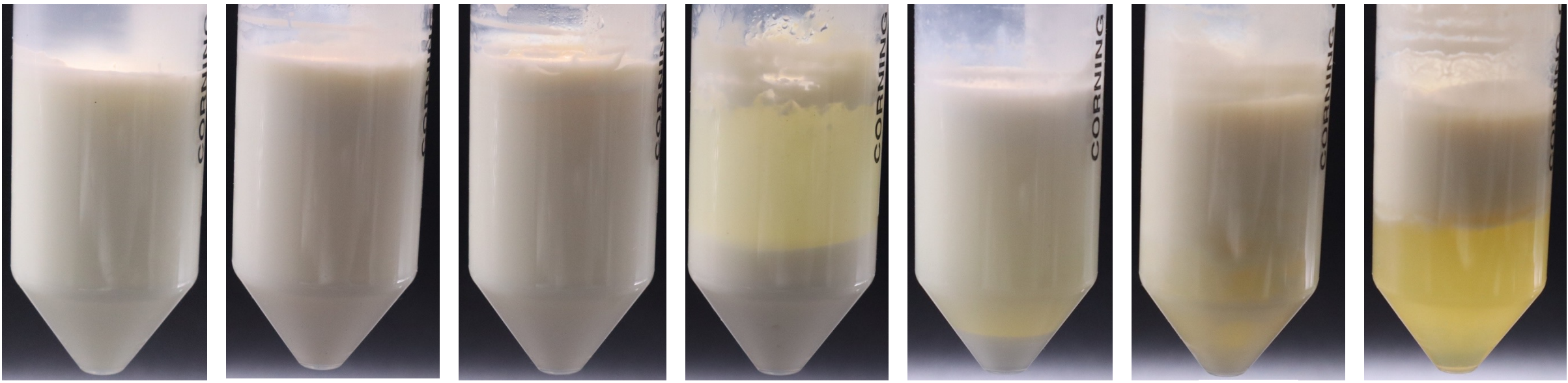


1000  $\mu\text{U}/\text{mL}$   
4 wks

100  $\mu\text{U}/\text{mL}$   
12 wks

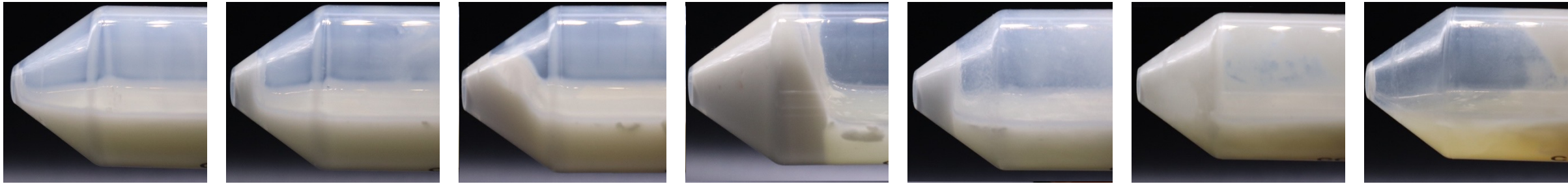
1000  $\mu\text{U}/\text{mL}$   
12 wks

at higher protease levels,  $\alpha$ - and  $\beta$ -caseins  
are also targeted



4 °C

22 °C



Up to 1 year  
Any level of  
AprX activity

10 days

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# Factors Influencing AprX Activity in Milk

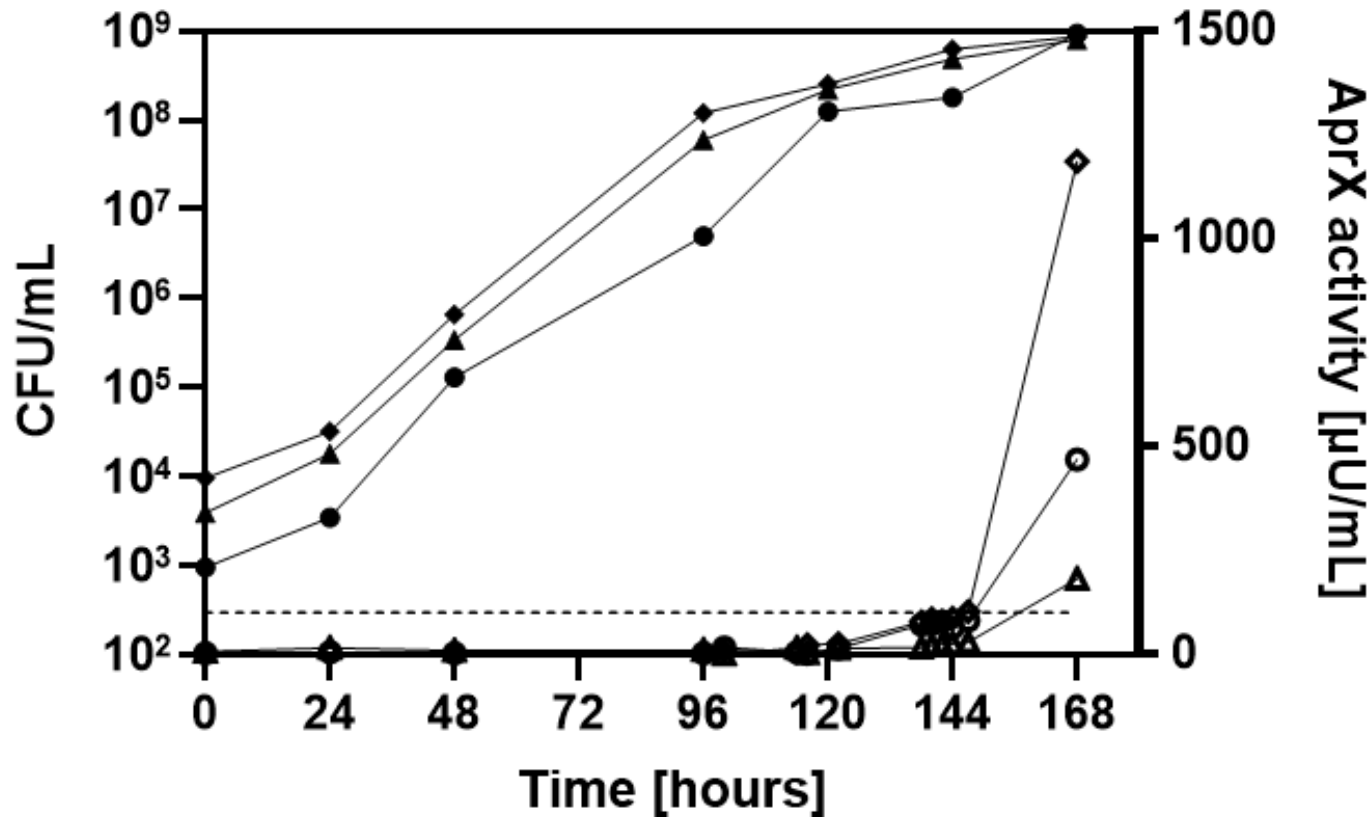
1. The planktonic density (cfu/mL) of AprX-secreting Pseudomonads
2. The presence of AprX-secreting biofilms of Pseudomonads
3. The dilution of AprX-contaminated milk with “clean” milk
4. Partial inactivation of AprX by heat treatment

# Factors Influencing AprX Activity in Milk

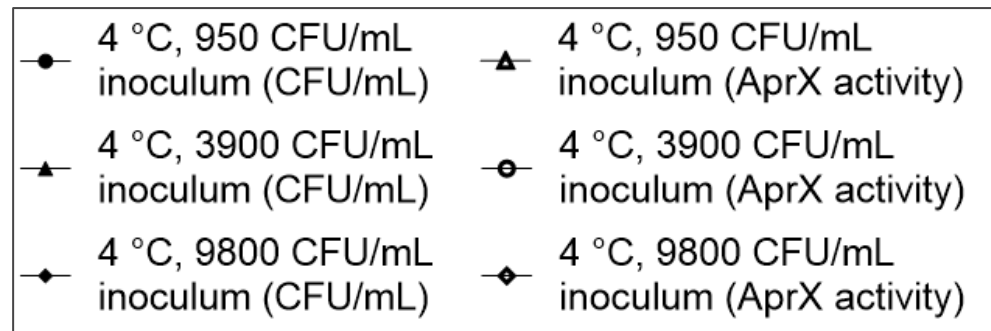
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# *P. fluorescens* (Strain 65) Growth & AprX secretion

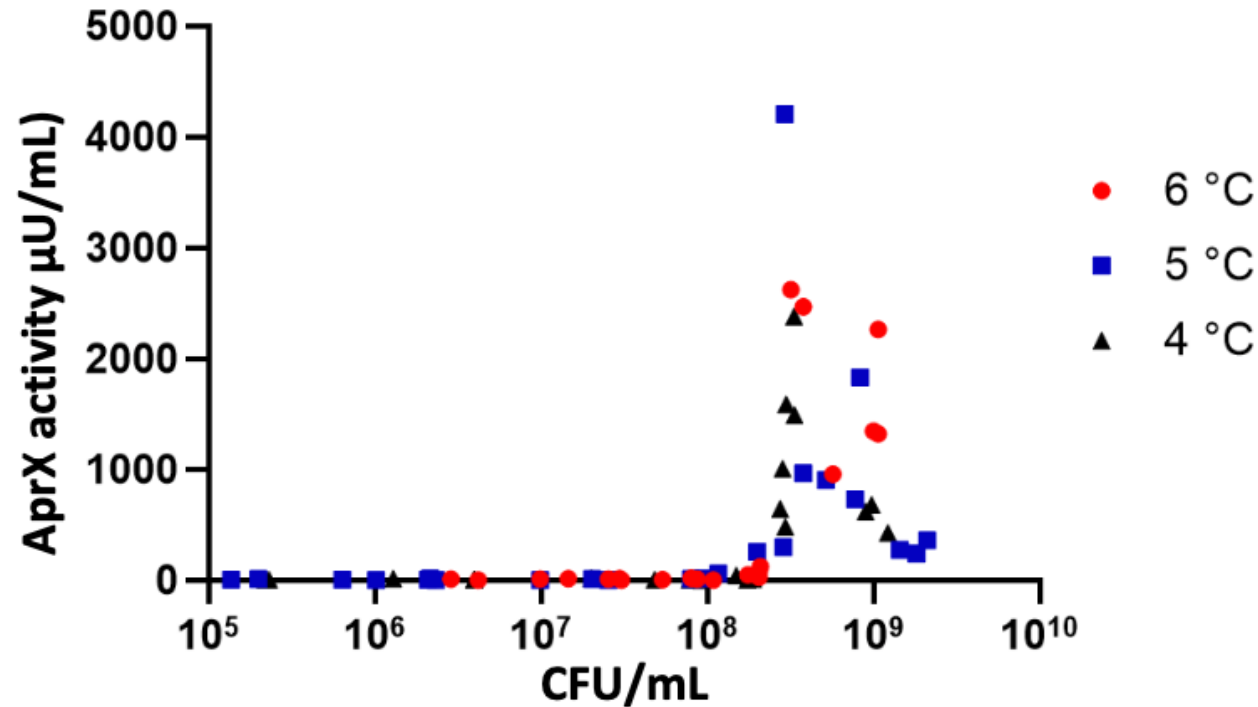


Full cream UHT milk @  
4°C with gentle shaking



# *Pseudomonas* cell density and AprX activity

We inoculated milk samples with low levels of *P. fluorescens*, incubated them at 4-6 °C and followed the time course of bacterial growth and AprX secretion (Fig. 3).



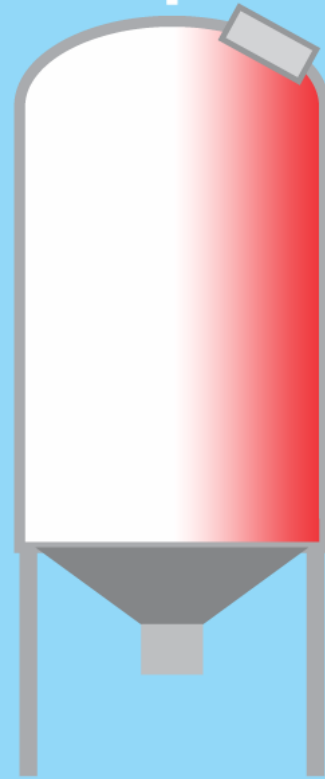
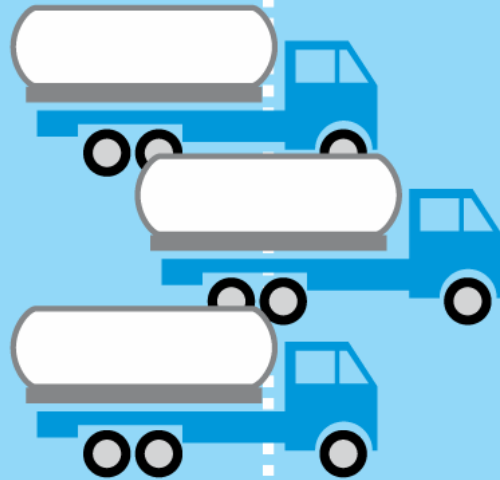
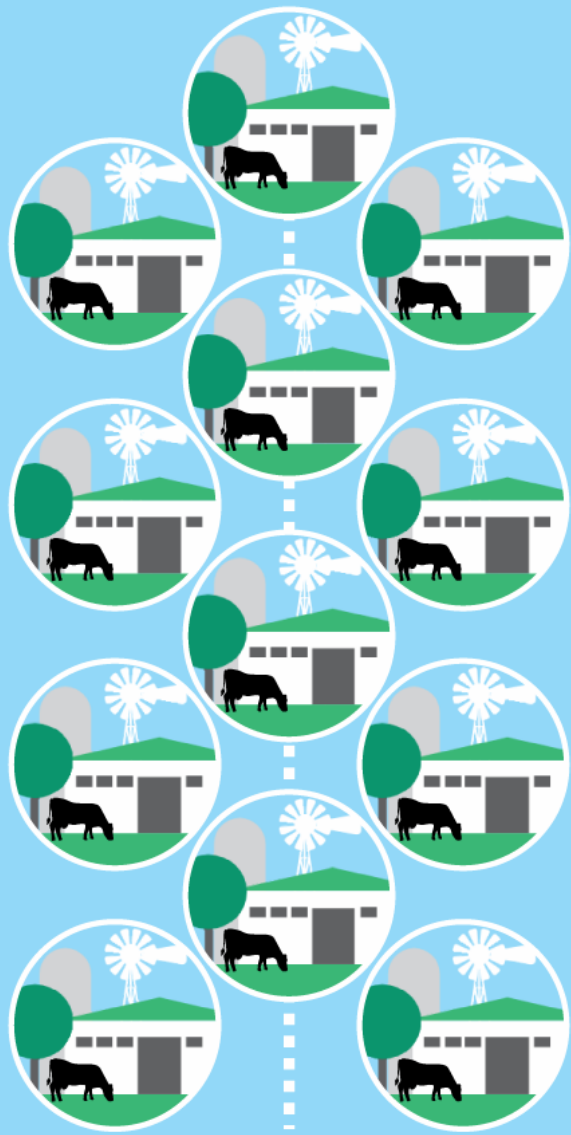
**Figure 3.** AprX secretion by *Pseudomonas fluorescens* in milk. Correlation between AprX activity detected directly in milk samples with CFU/mL counts of *P. fluorescens*.

FARMS

TANKERS

SILO

FINISHED  
PRODUCT



UHT  
process



Bacterial growth

AprX destruction

# Factors Influencing AprX Activity in Milk

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# Biofilms as a Source of AprX

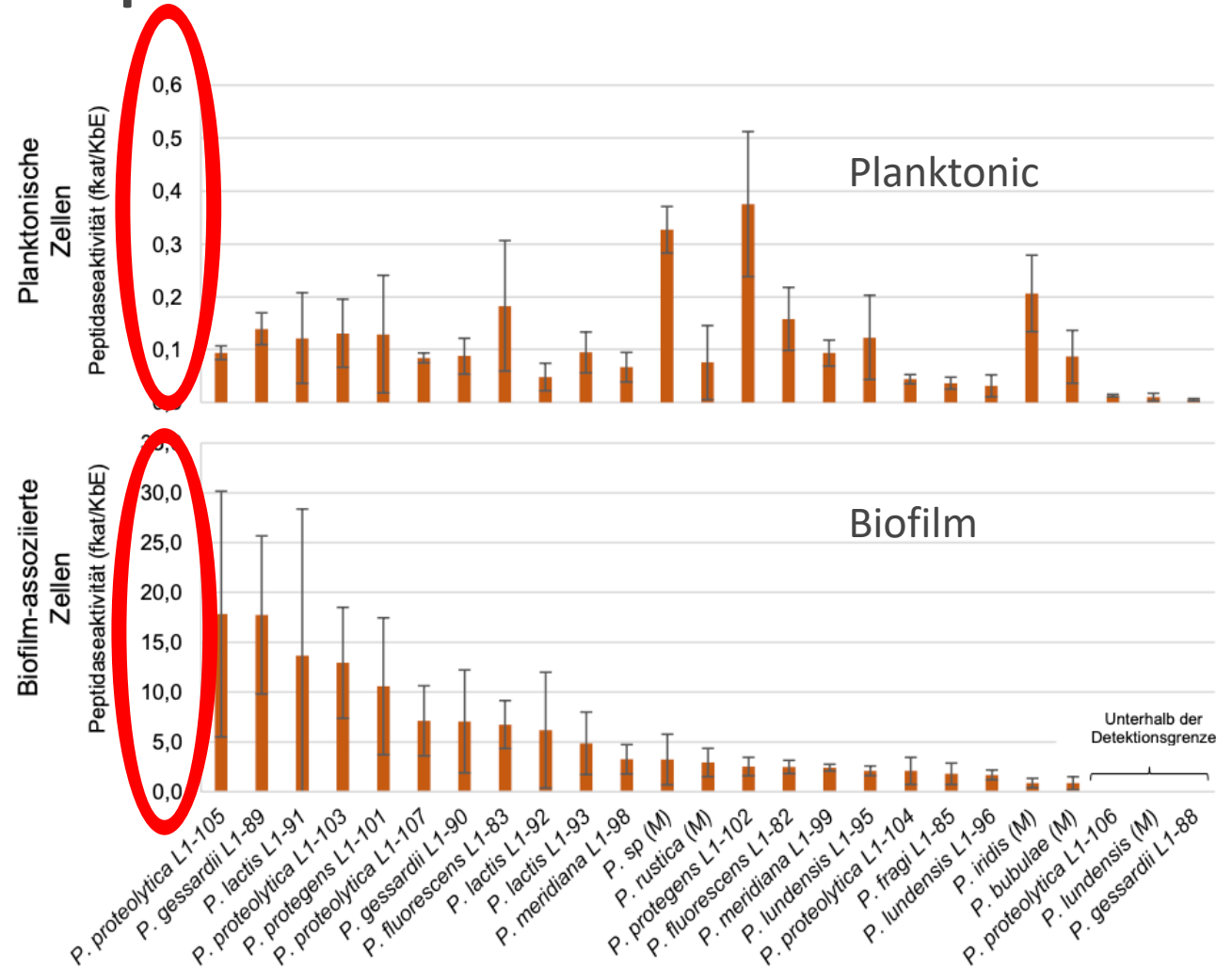
“ ...the formation of peptidases was detected in *Pseudomonas* mono-biofilms at a higher level when compared to planktonic growth ...

high *Pseudomonas* counts [detected] on seals made of rubber, e.g., the spigot, the tank lid and the teat cup collector, indicate favored growth of biofilms at these sites.”

Stefanie Gieschler

*Pseudomonas* spp. in North German raw milk: Determination of entry routes, Characterization of biofilm formation and peptidase activity.

Ph.D Thesis, University of Kiel, 2022



# Factors Influencing AprX Activity in Milk

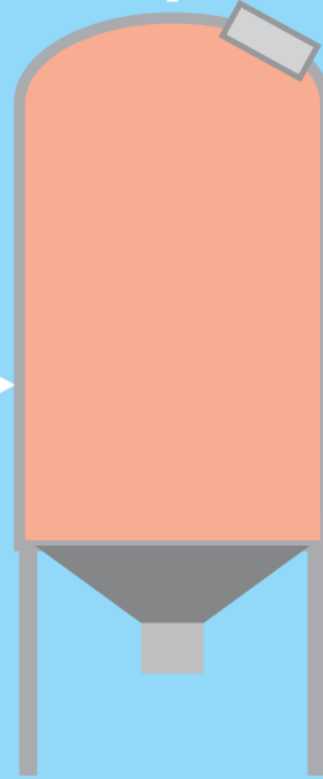
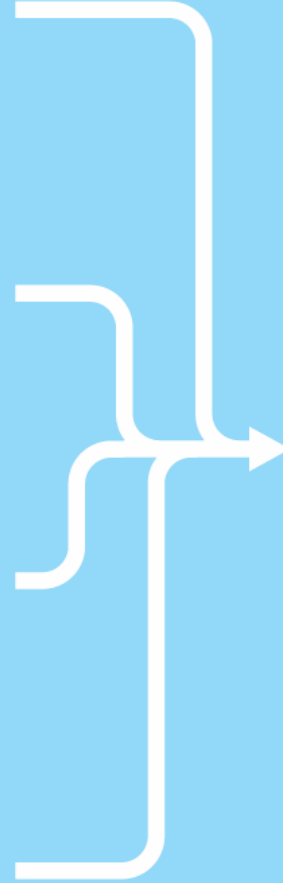
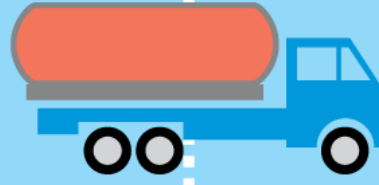
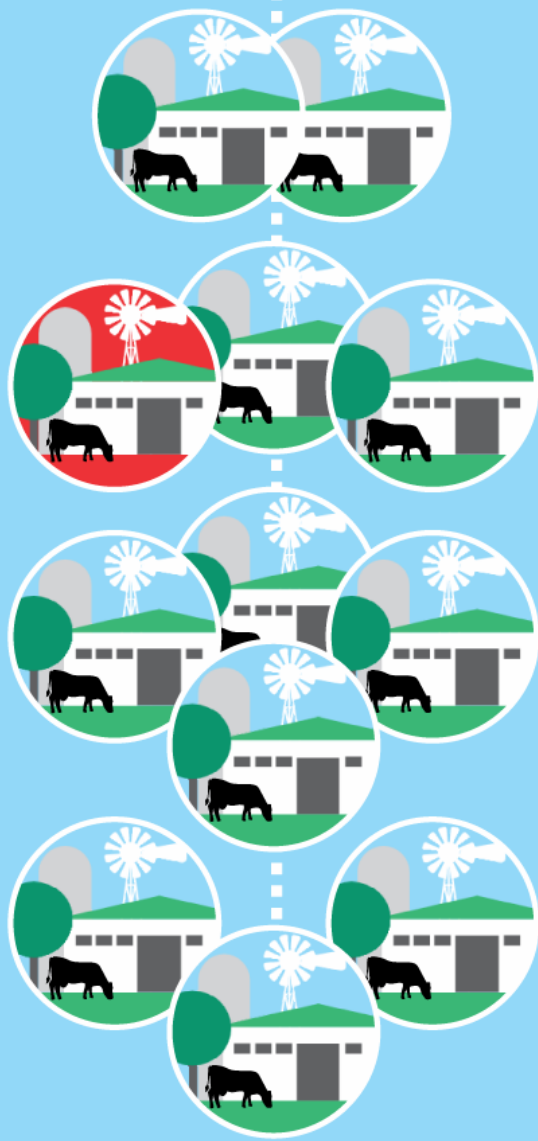
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FARMS

TANKERS

SILO

FINISHED  
PRODUCT



UHT  
process



Protease dilution

AprX destruction



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International Dairy Journal

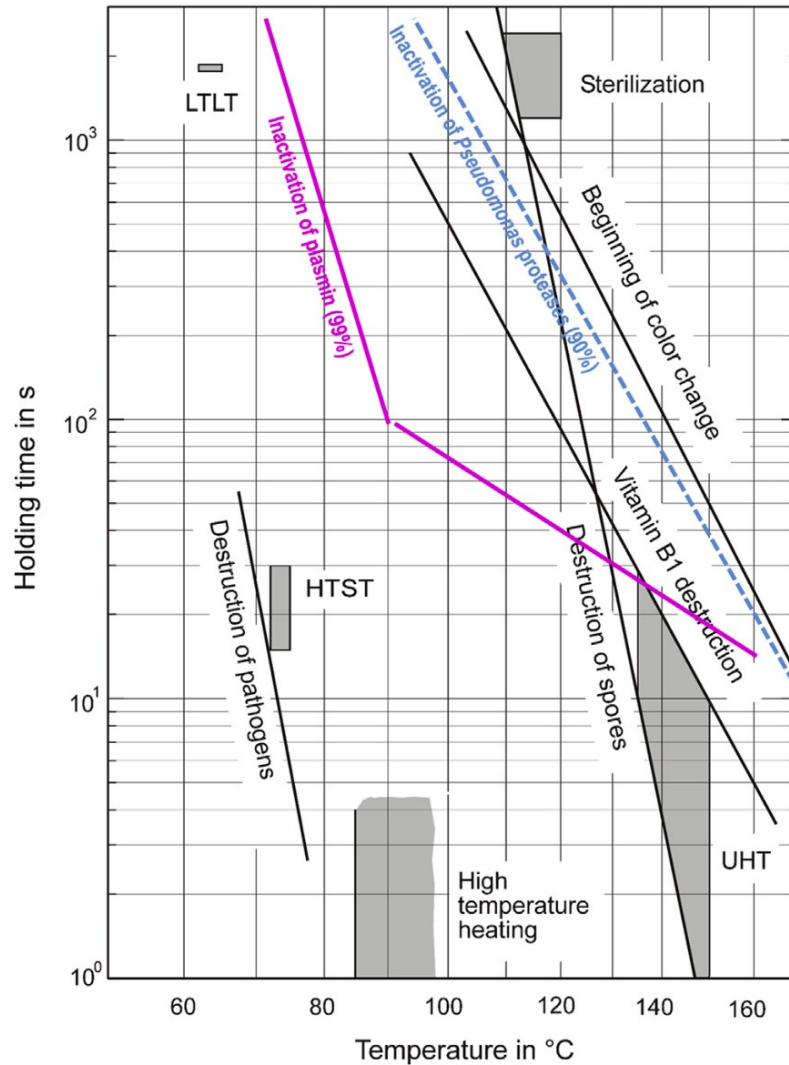
journal homepage: [www.elsevier.com/locate/idairyj](http://www.elsevier.com/locate/idairyj)



Review

## Heat stability of indigenous milk plasmin and proteases from *Pseudomonas*: A challenge in the production of ultra-high temperature milk products

Marina Stoeckel <sup>a,\*</sup>, Melanie Lidolt <sup>a</sup>, Timo Stressler <sup>b</sup>, Lutz Fischer <sup>b</sup>, Mareike Wenning <sup>c</sup>, Jörg Hinrichs <sup>a</sup>



**Fig. 1.** Inactivation of plasmin by 99% (solid line) and of the proteases from *Pseudomonas* by 90% (dashed line) in milk (modified from Kessler, 2002). The mean values from Tables 1 and 2 were used for the kinetic data.



# Factors Influencing AprX Activity in Milk

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# Protease Activity Levels Before and After UHT

	Measured before UHT	% removal during UHT	Residual after UHT
AprX	10 $\mu$ U	70-90	1-3 $\mu$ U
Plasmin	10 mU	99*	0.1 mU
Subtilisin	10 $\mu$ U	50?	5 $\mu$ U

\* But note that inappropriate UHT settings can activate plasminogen leading to a 2-fold INCREASE in plasmin activity

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This is what causes the damage

\* But note that inappropriate UHT settings can activate plasminogen leading to a 2-fold INCREASE in plasmin activity

# To Minimise AprX Contamination in UHT

1. Minimise time and temperature for raw milk
2. Control biofilms to eliminate point sources of AprX
3. Measure protease as close to its potential source as possible
4. Apply optimal UHT time/temperature protocols

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# Current On-Site Tests Cannot Measure AprX

## ATP Luminescence



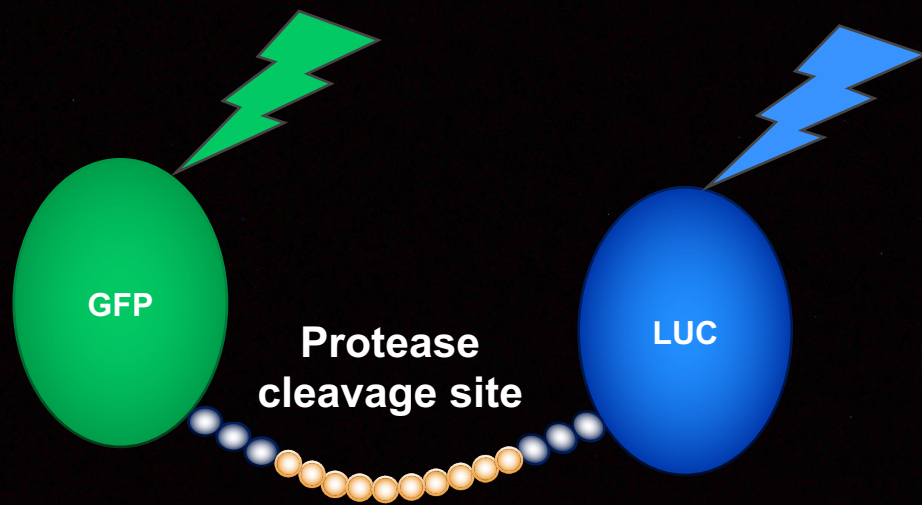
## Lateral Flow Immunoassay



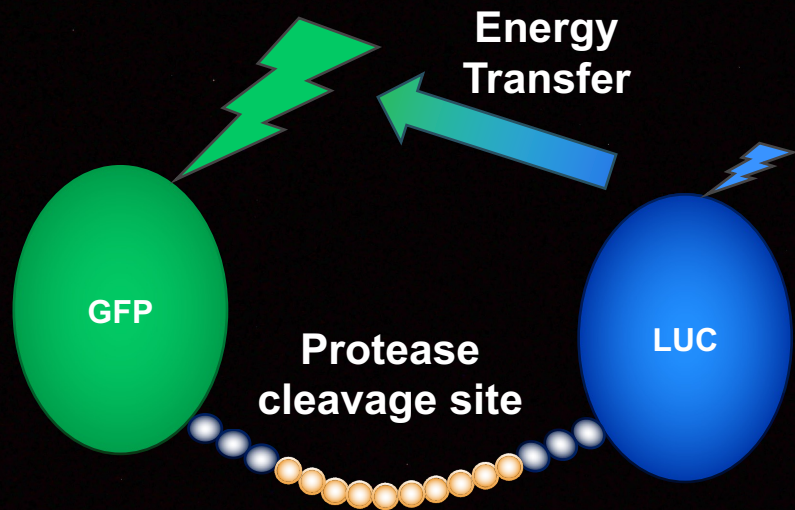
## Electrochemical Biosensors

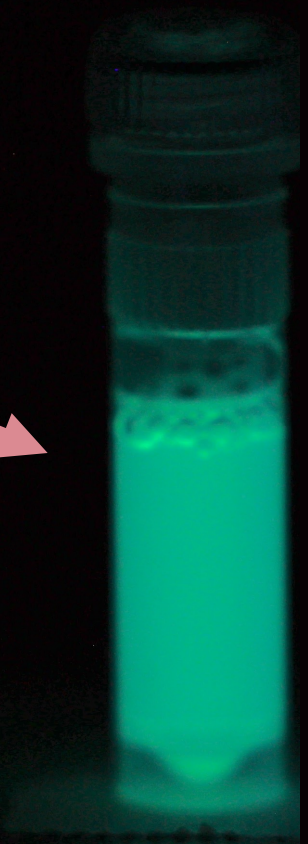
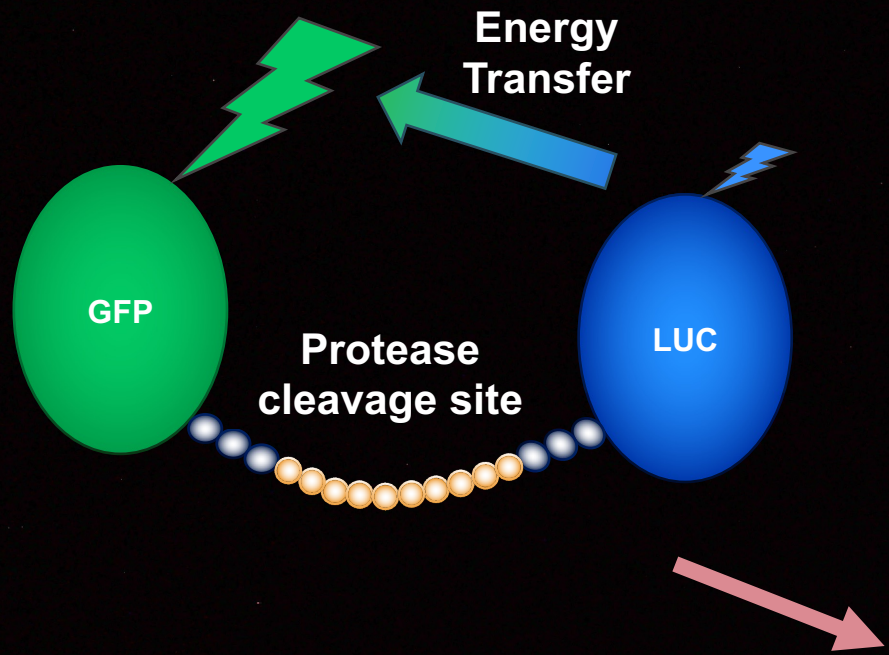


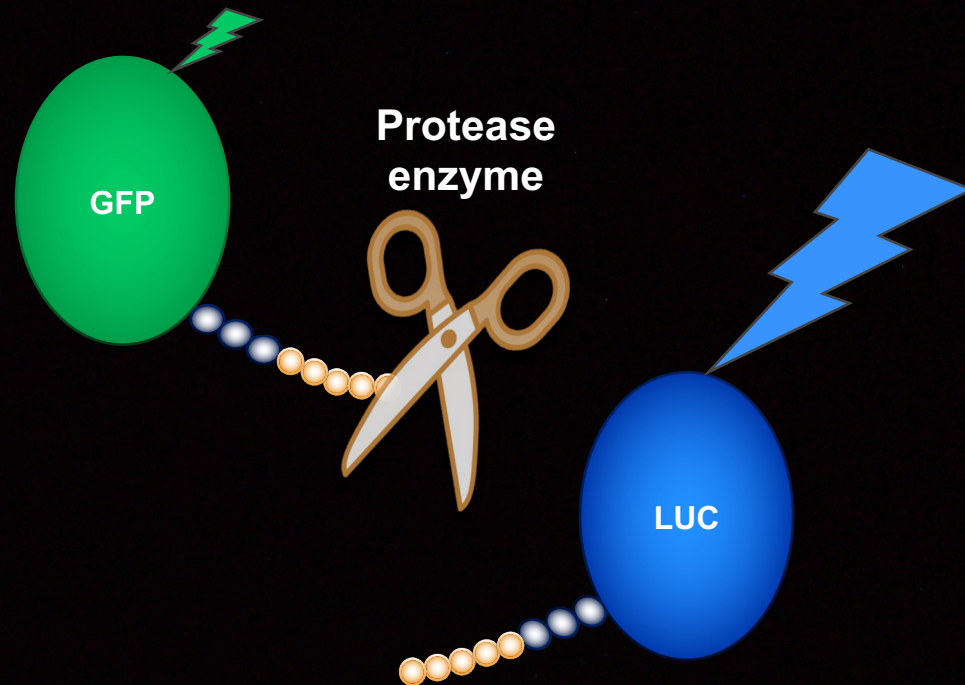
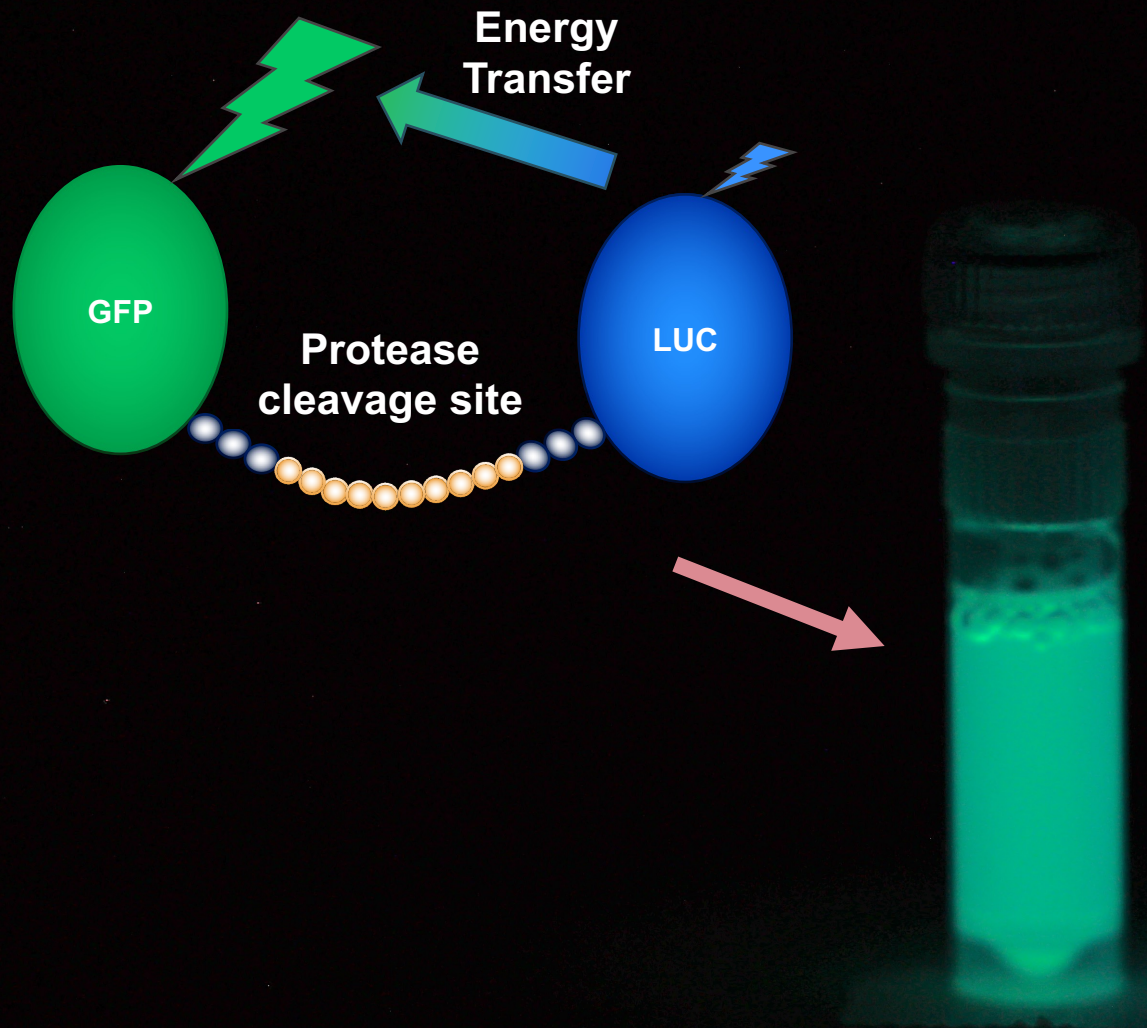
# CYBERTONGUE® Technology Application to Protease Testing

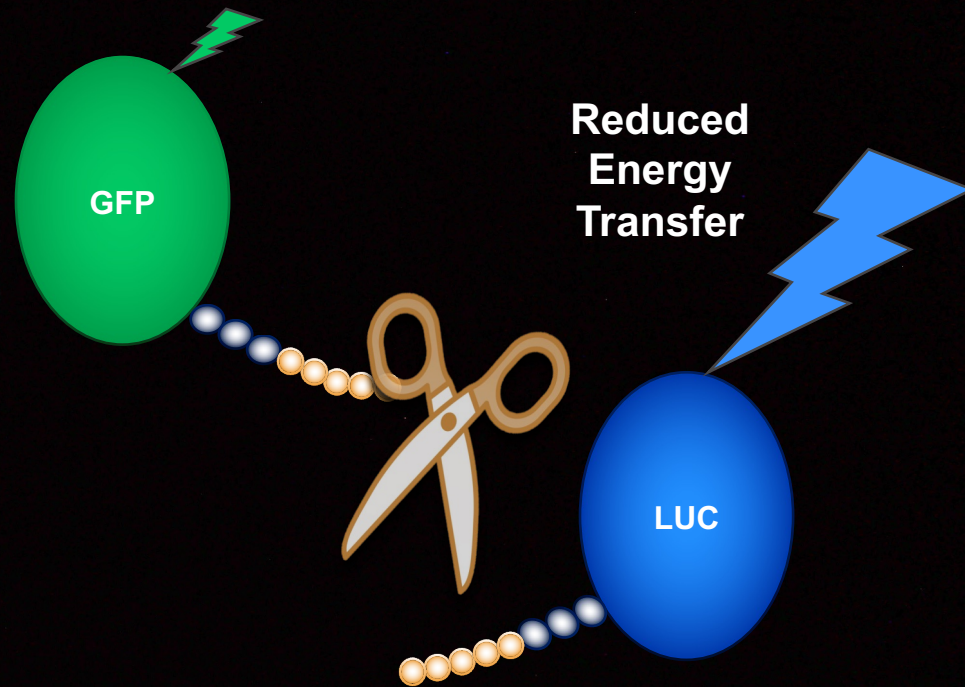
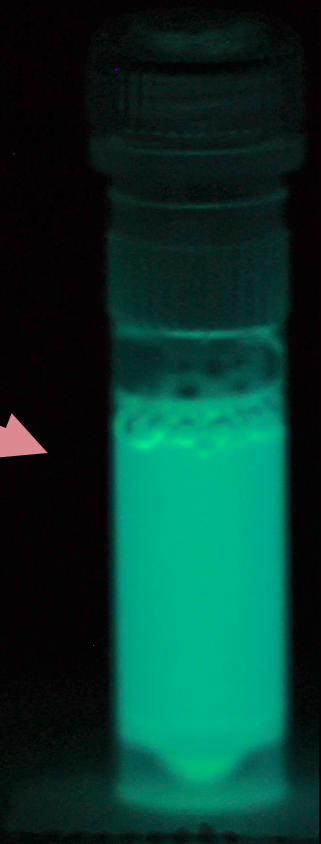
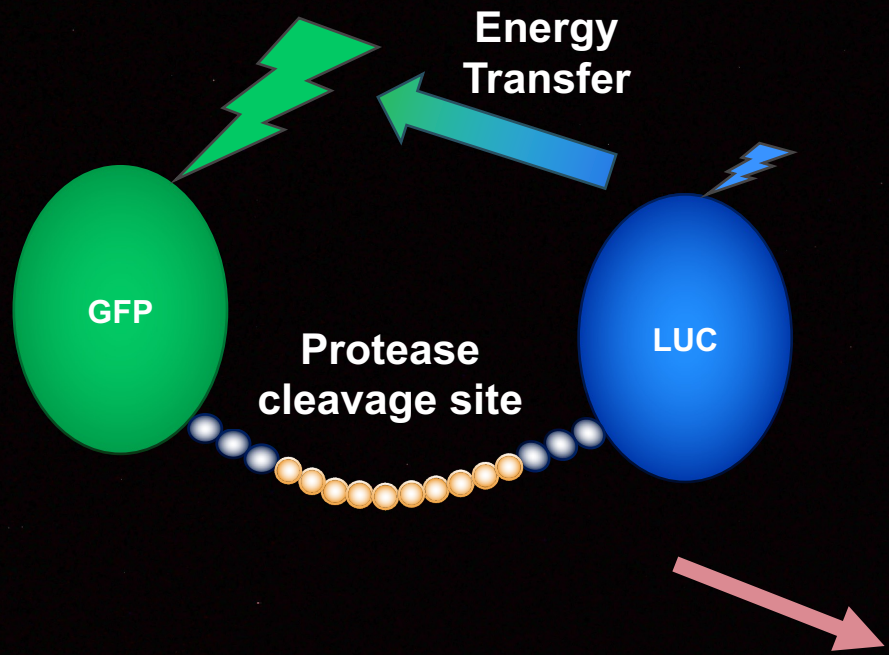


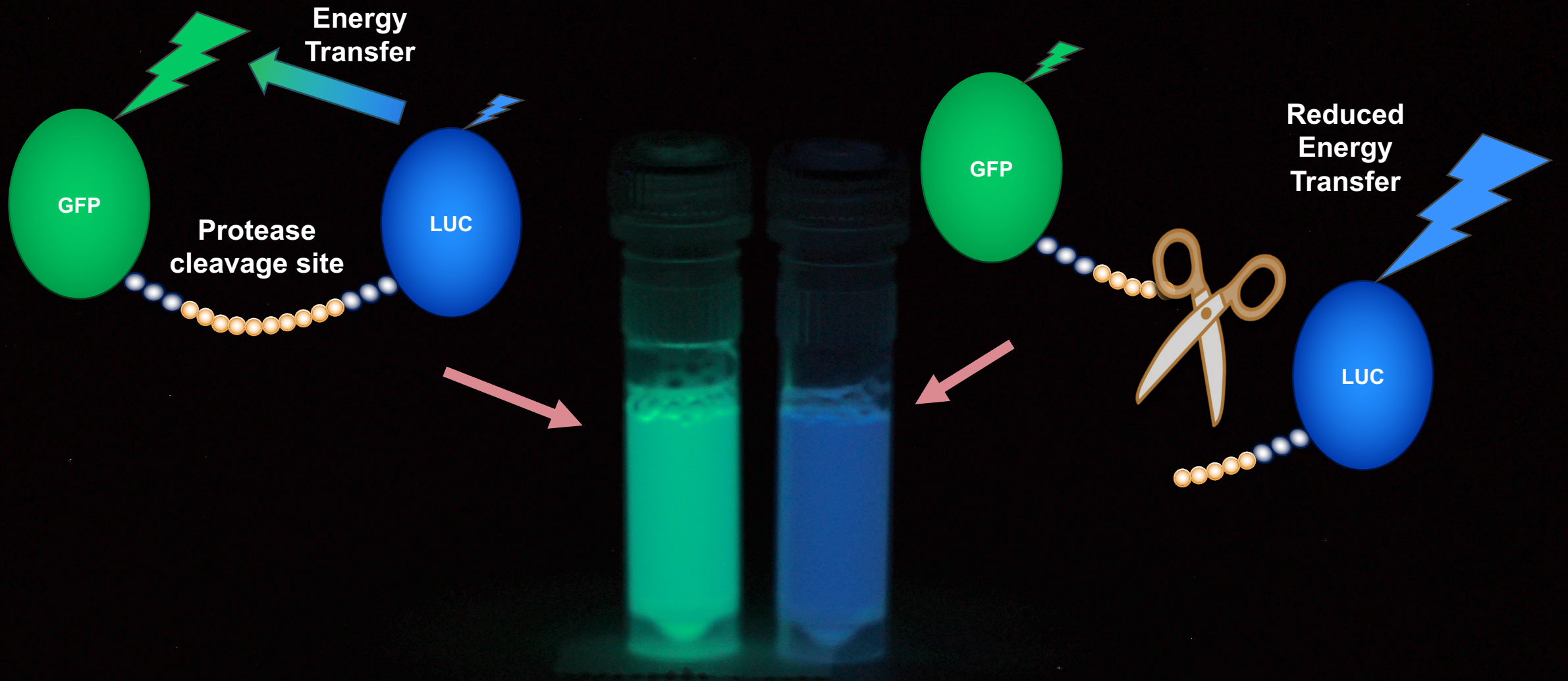


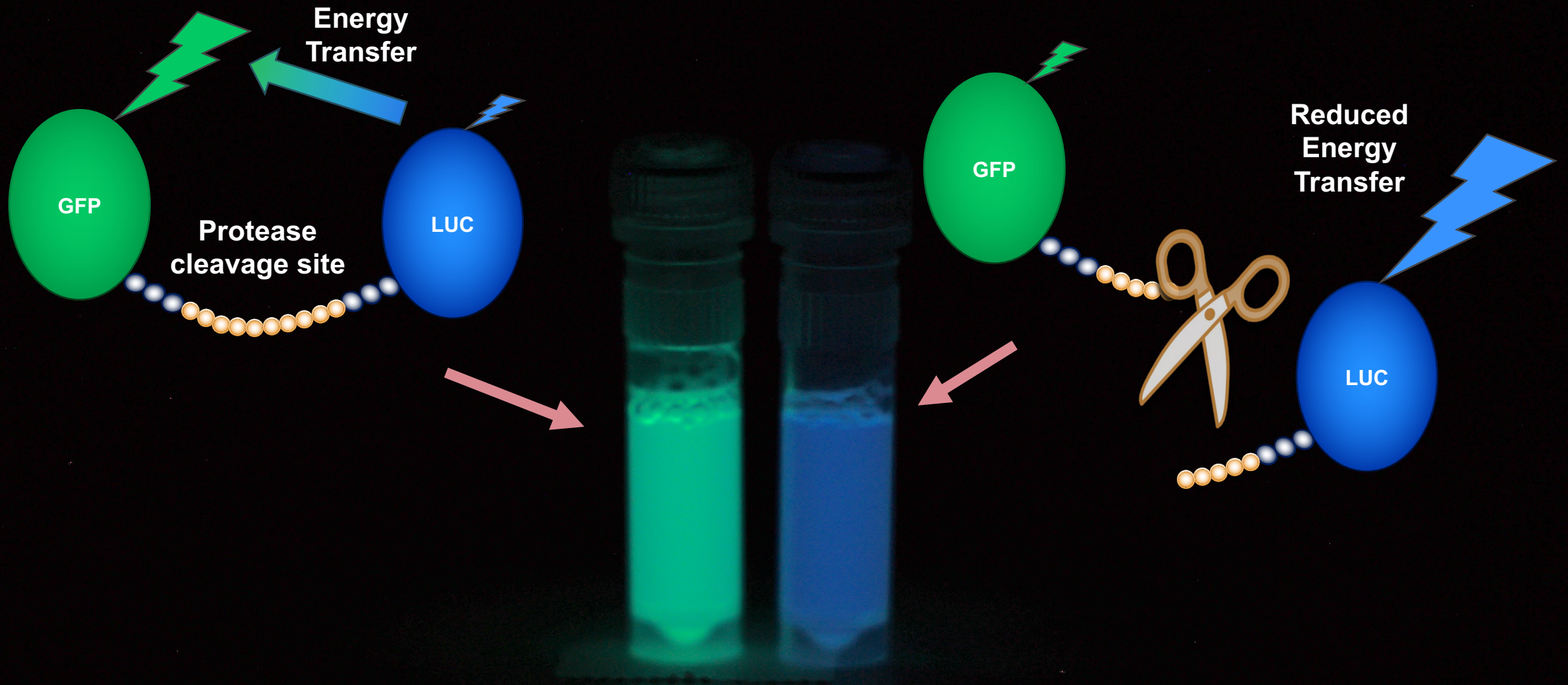










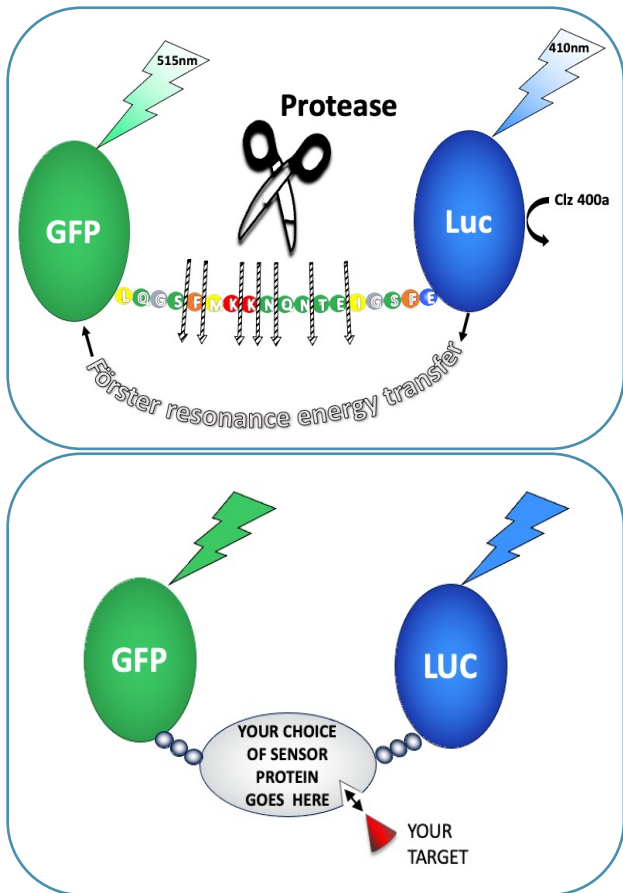


Bioluminescence Resonance Energy Transfer (BRET)

# The CYBERTONGUE<sup>®</sup> Technical Solution

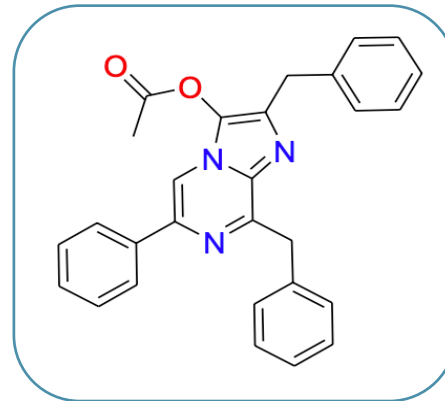
## A BRET-based Biosensor

Molecules designed and manufactured in-house



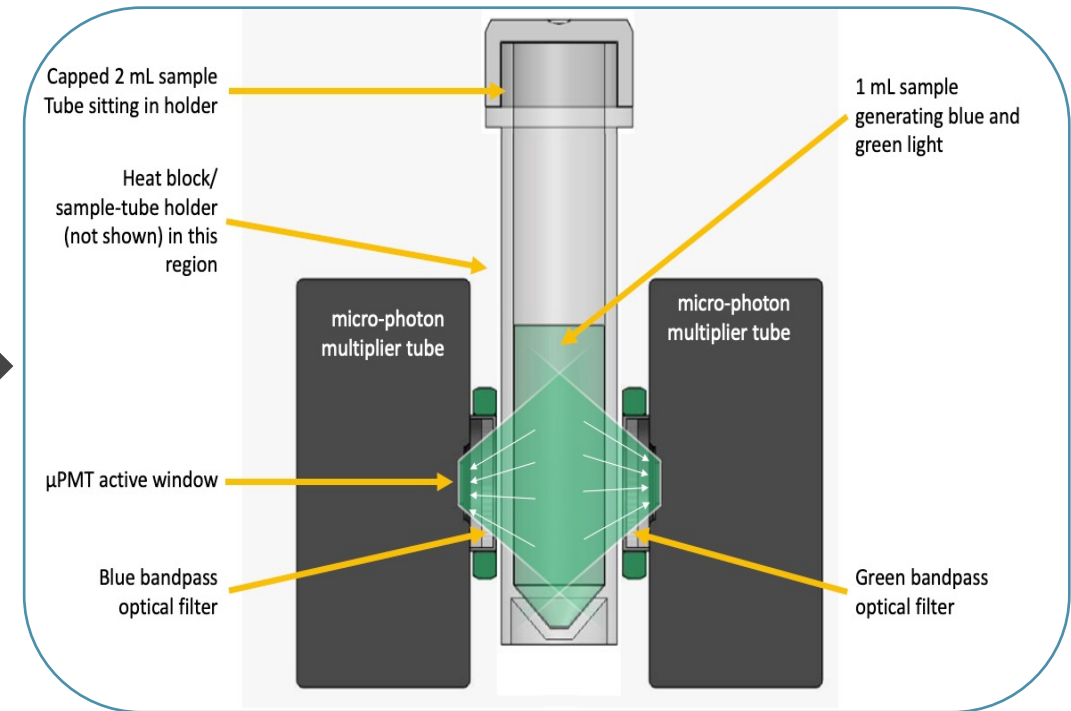
## A Stable Substrate

Custom-synthesised, then formulated by PPB Tech



## A State-of-the-art Device

Specified by PPB Tech, designed and built under contract



Link to video demo:

[https://youtu.be/v\\_CTbFMPEG4](https://youtu.be/v_CTbFMPEG4)

cream of tartar  
assay tube using the  
ed volume pipette.

next

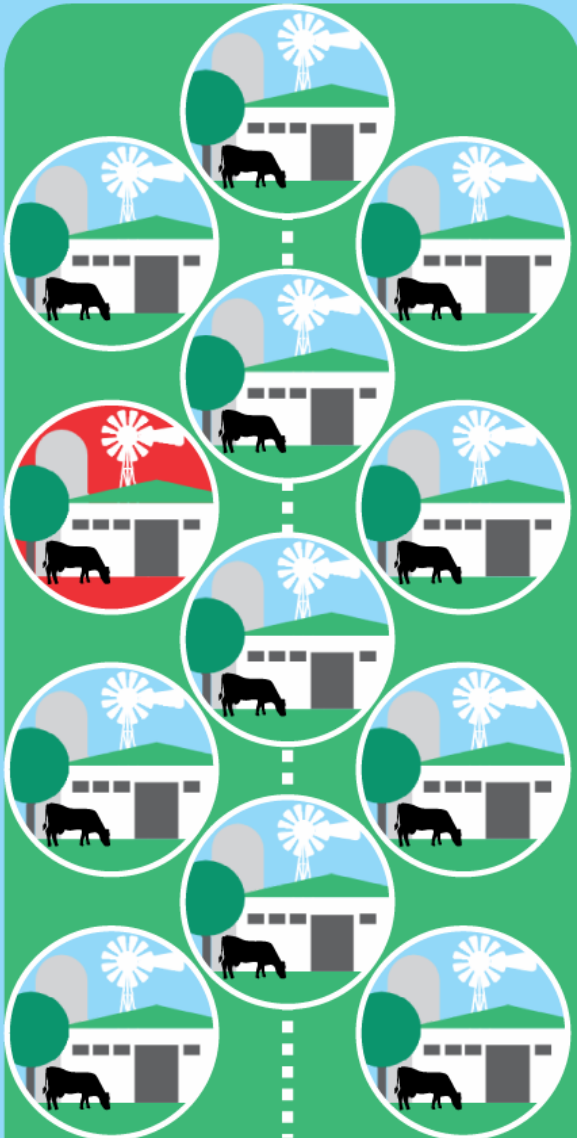
back





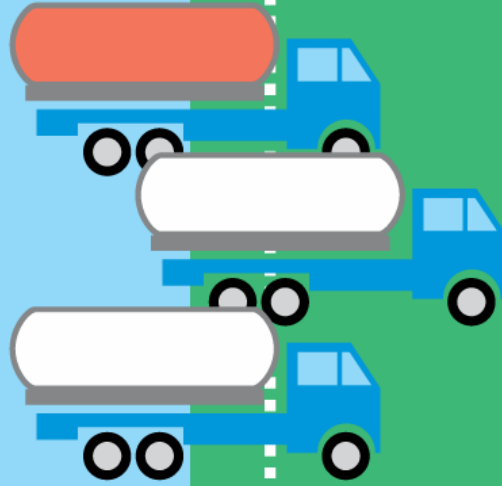
# Control Points for Measuring AprX

# FARMS



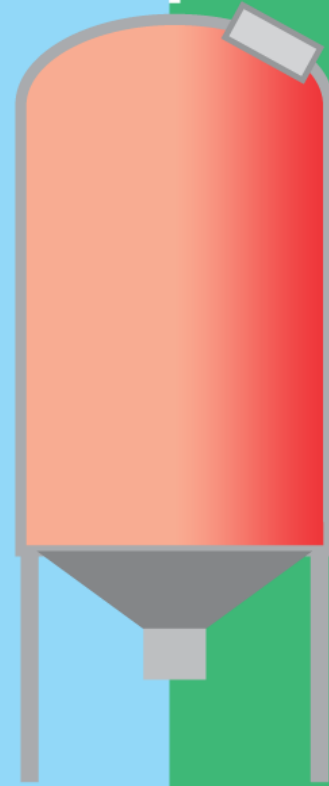
CP1

# TANKERS



CP2

# SILO



CP3

UHT process

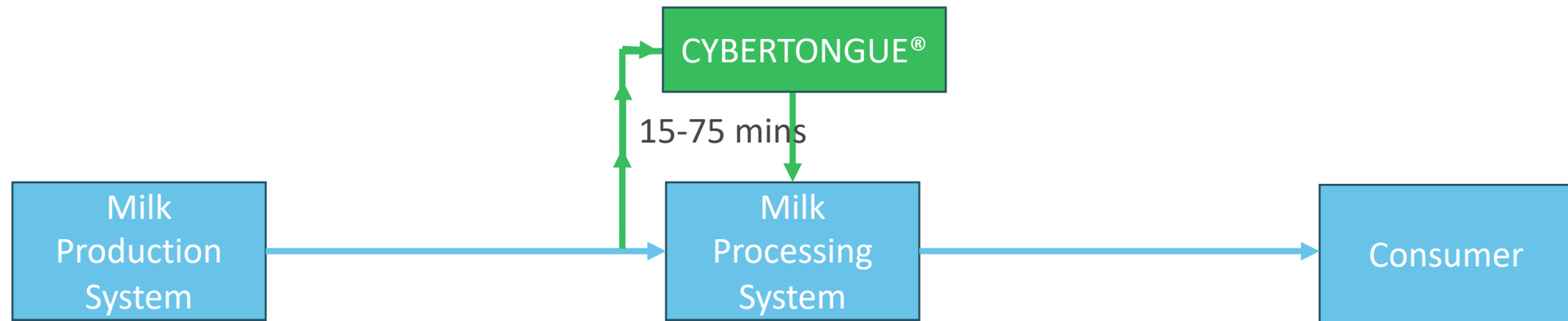
# FINISHED PRODUCT



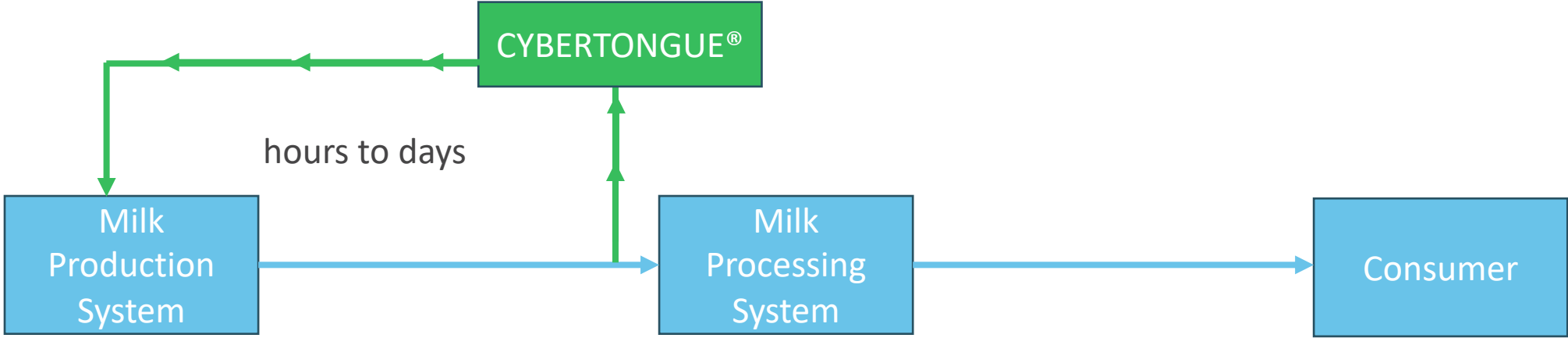
# Rapid Control Loop Using CYBERTONGUE®



# Rapid Control Loop Using CYBERTONGUE®



# Traditional Feedback Loop Using CYBERTONGUE®



quality results  
in minutes,  
not days.

HUMAN HEALTH  
AGRICULTURE  
FOOD PROCESSING



**CYBERTONGUE®**  
Combines ultra-sensitive biosensors  
inspired by nature applied on a compact  
detection device, providing customers  
the means to generate their own  
laboratory quality test results.



Laboratory Quality Results in  
Minutes

PPB Tech  
The CYBERTONGUE®  
Company

