Afterlife of bacterial cell debris: Peptidoglycan in the gastrointestinal tract

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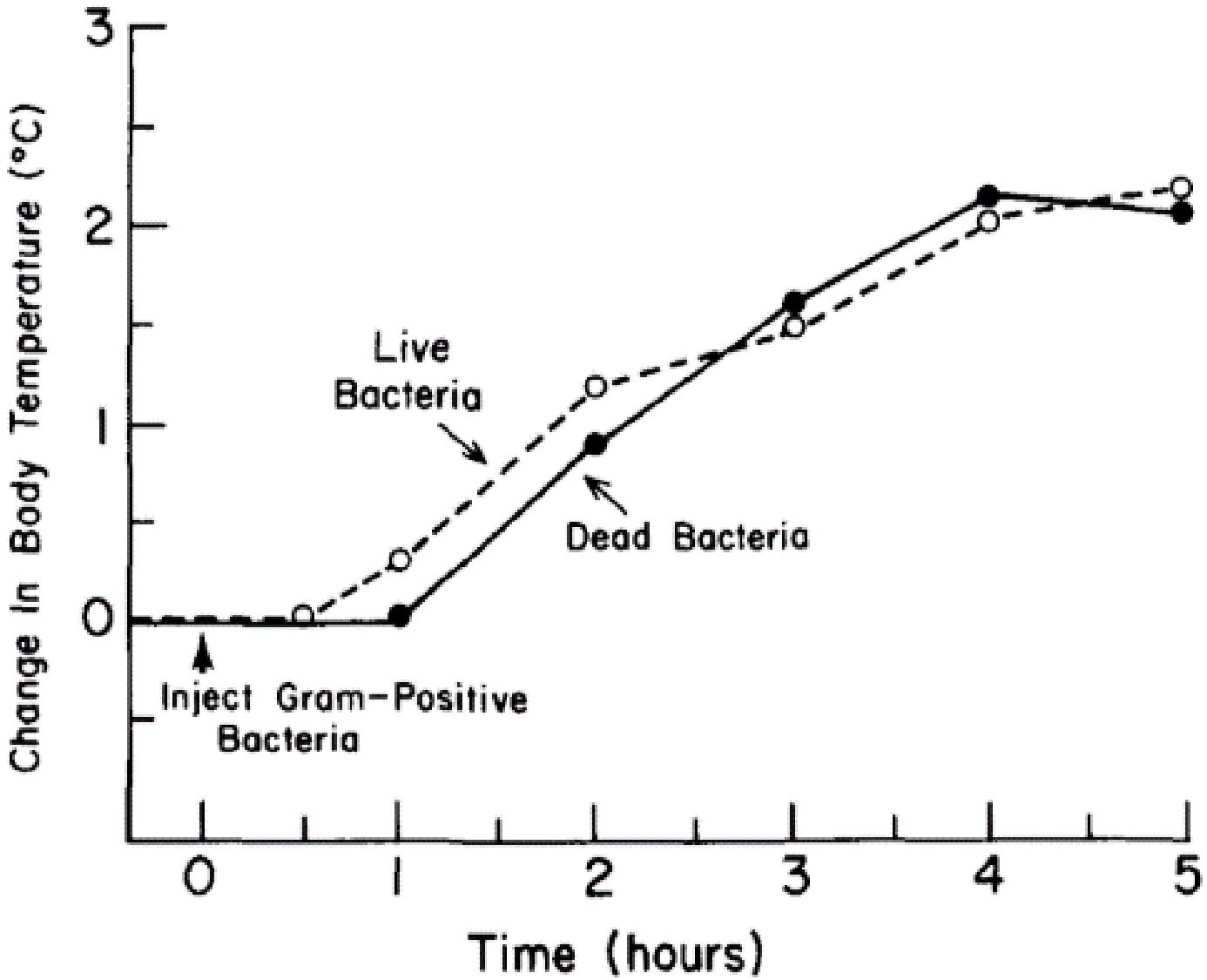
Outline

1. The problem: dead bacteria and cell debris in the gut
2. Solving the problem: using a novel microbial muramidase
3. How the solution works

Life after death

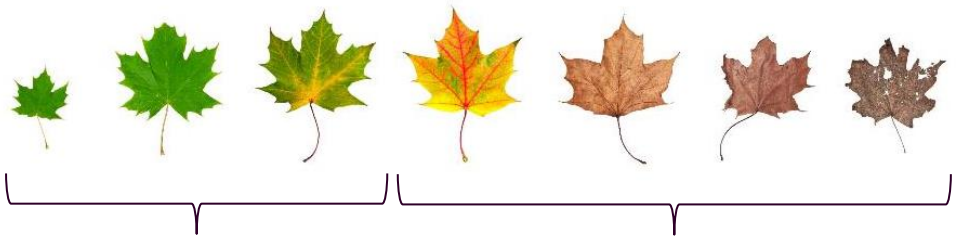


Nikolai Fedorovich Gamaleia (1859-1949)

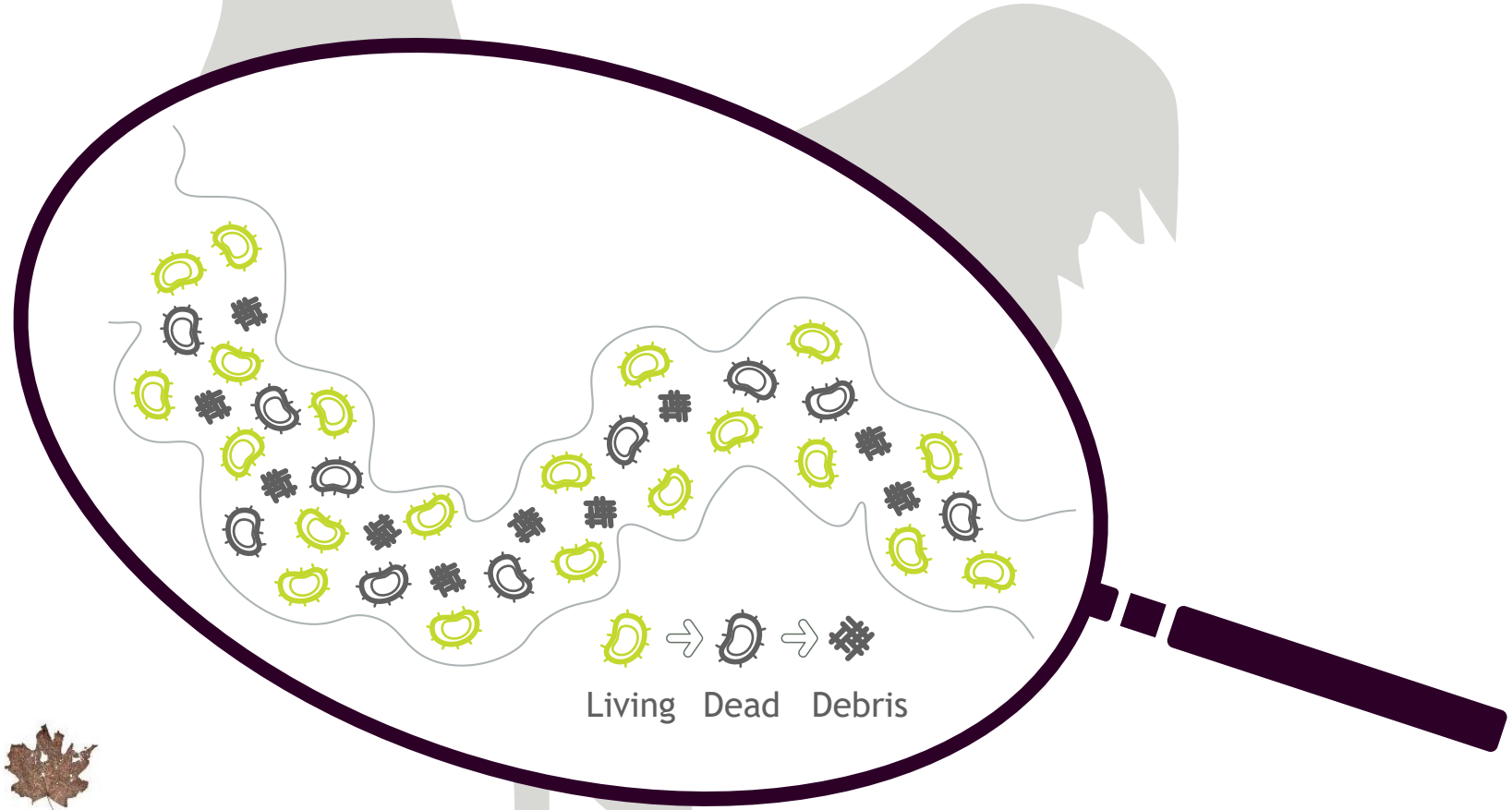
- Worked in Pasteur's lab in 1886
- Later joined Ilya Mechnikov
- studied the effect of injection of dead bacteria in rabbits and sheep

Matthew J. Kluger 2015 Fever: Its biology; Evolution, and Function, Jean-Marc Cavaillon and Mervyn Singer Inflammation 2018 Wiley-VCH Verlag GmbH & Co

Afterlife of bacterial cell debris



Live bacteria + Dead bacteria
(whole cells + fragments)



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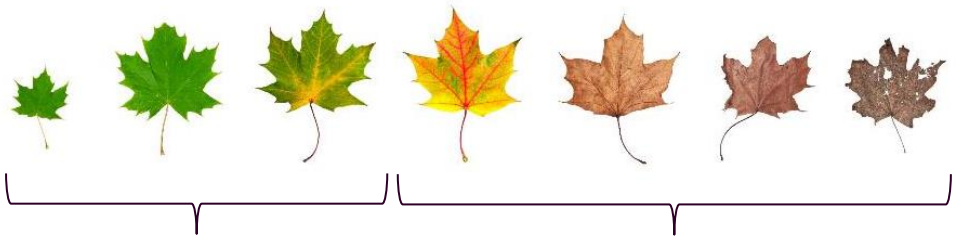
A holistic view of microbiota: includes dead bacteria and cell debris



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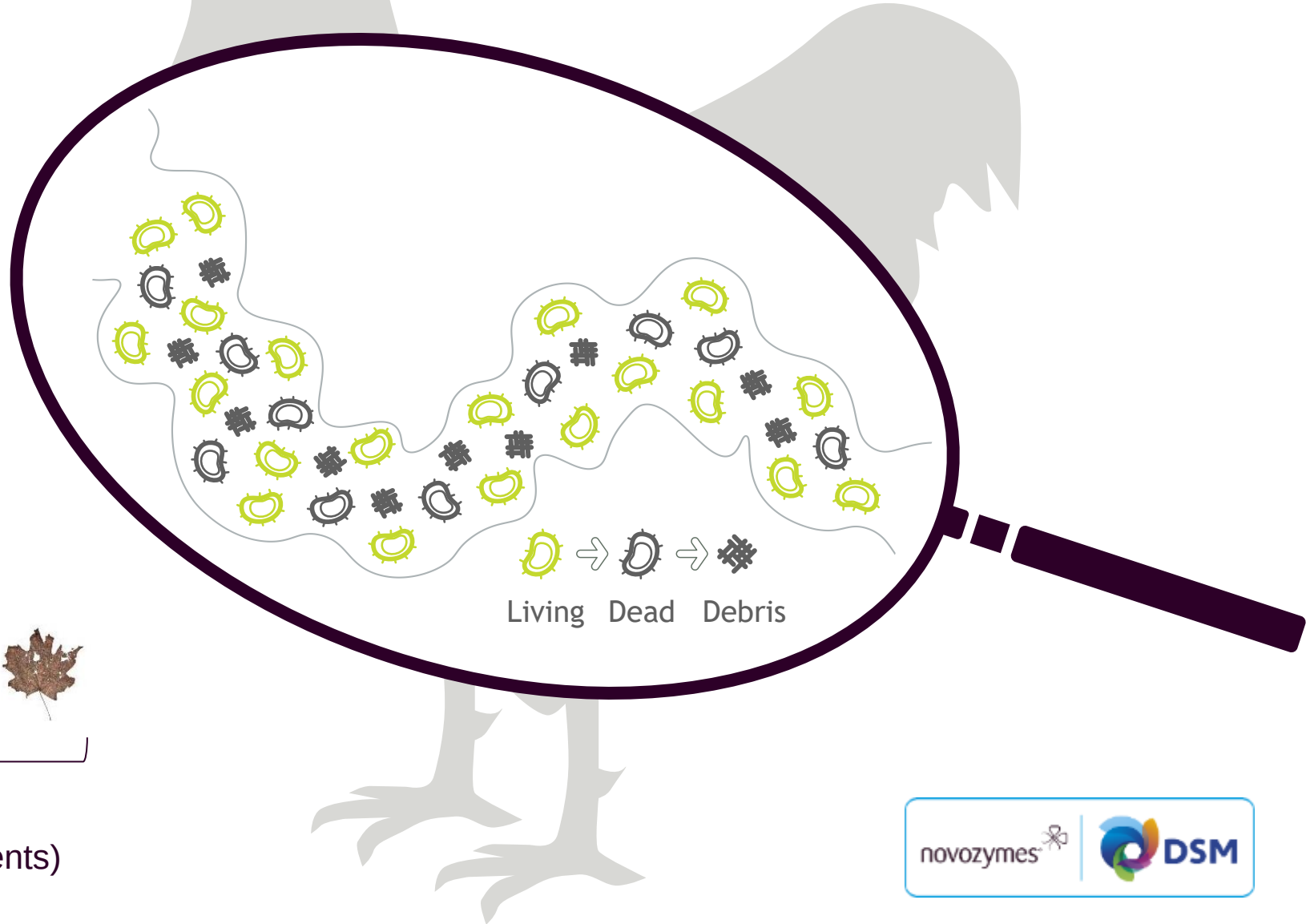
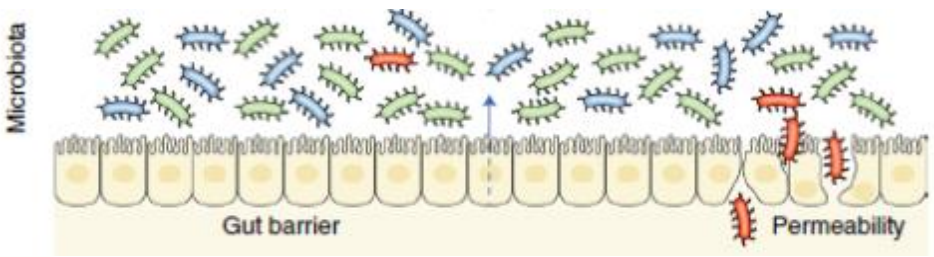
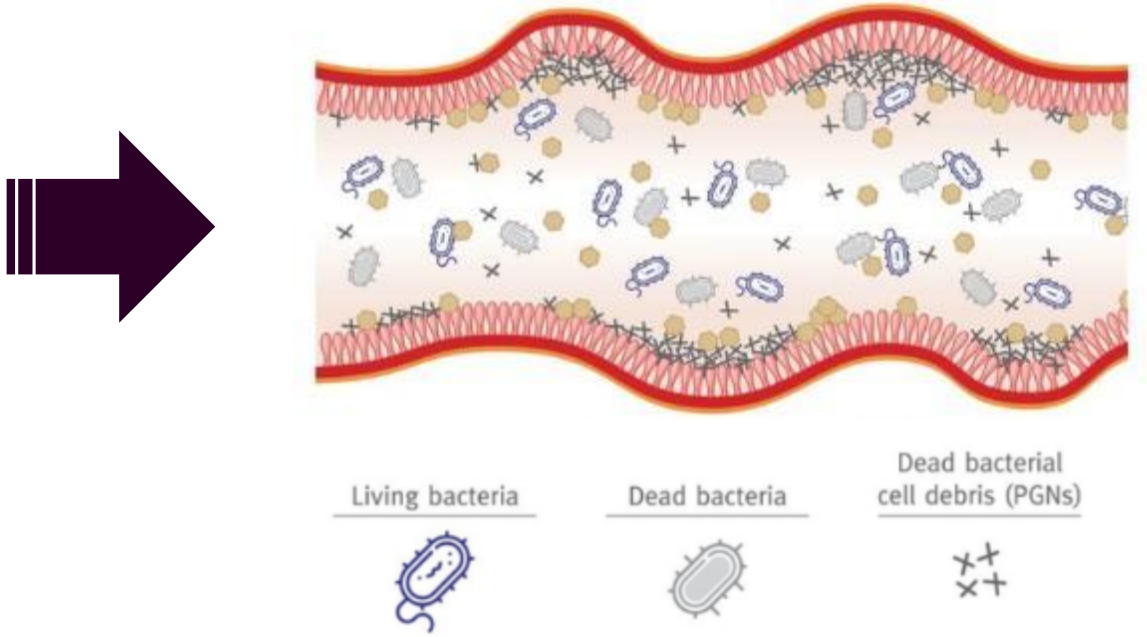


The problem: dead bacteria and bacterial cell debris in the gut

All parts interact with the host cells in the GIT

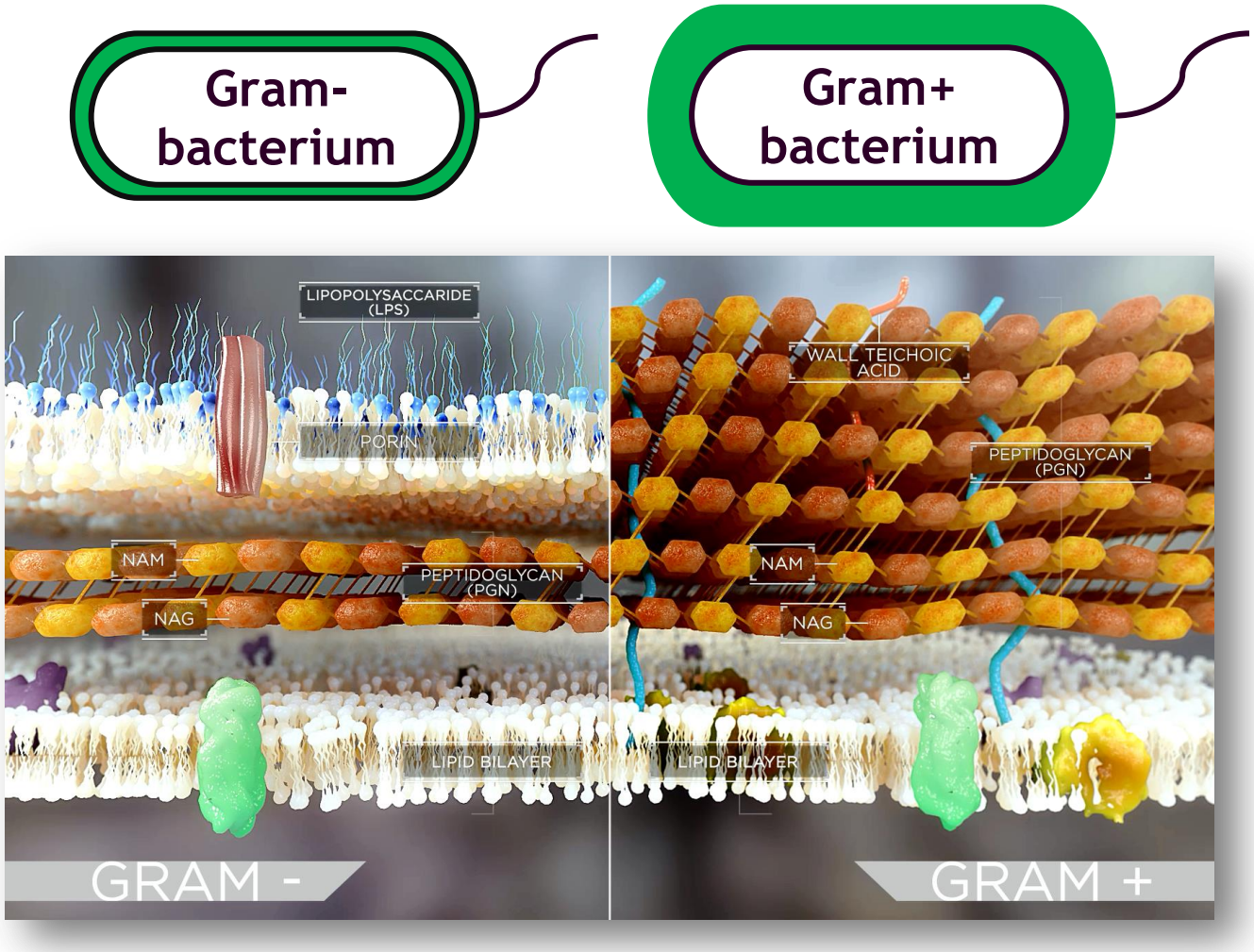




Feedstuffs Sep 26, 2018 Understanding threats to poultry performance, Nature Metabolism Jan 2019, Vol 1 34-46

Peptidoglycan (PGN): a major component of all bacteria

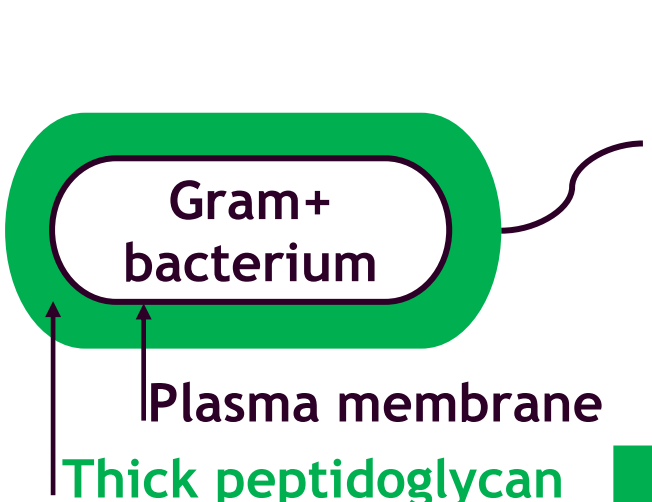
- PGN dry weight:
  - Gram- 10%
  - Gram+ 80-90%
- Unique biopolymer only found in bacterial cell wall
- Provides structure, shape and counteracts osmotic pressure
- Abundant in the gut



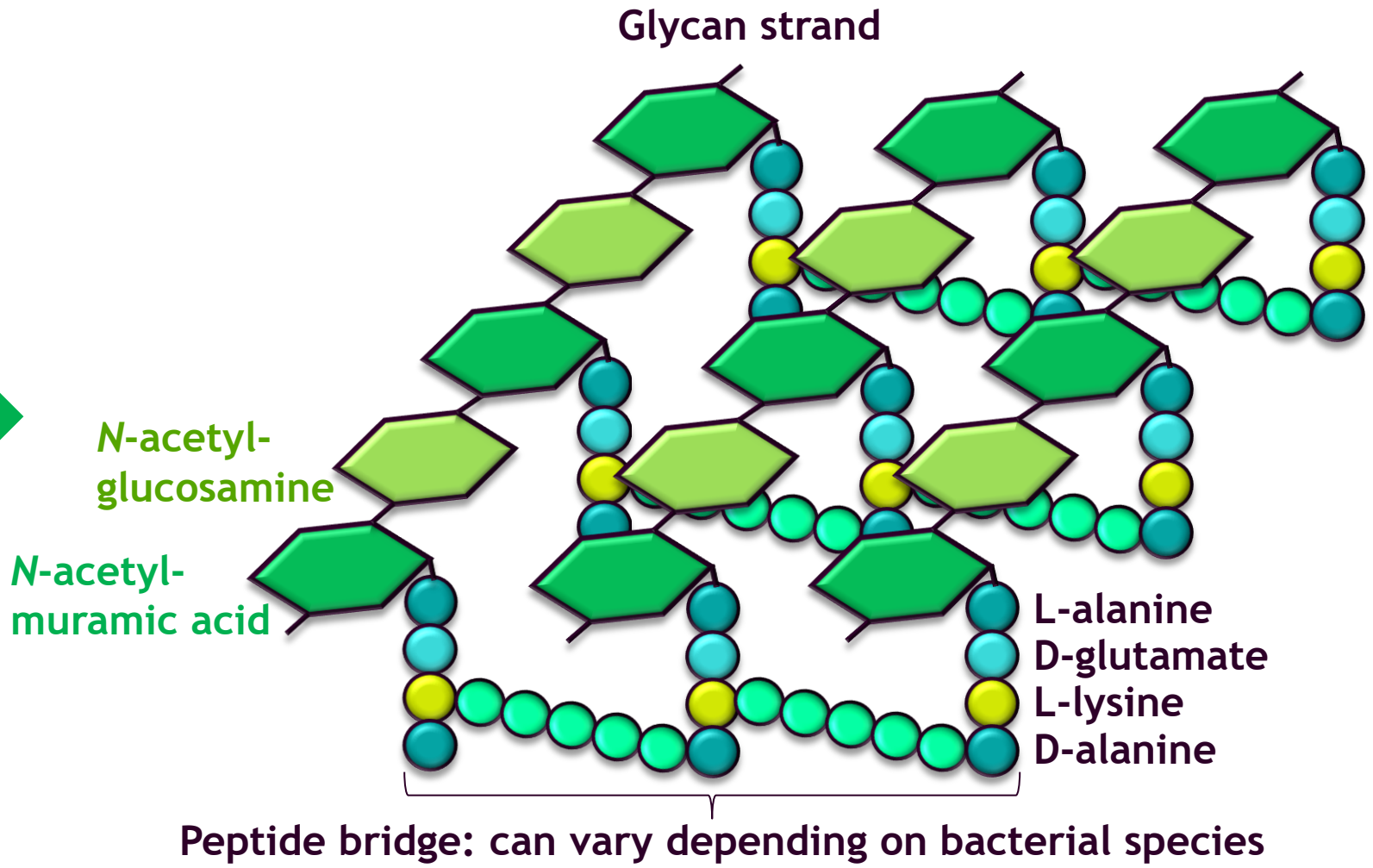
Peptidoglycan: a complex polymer forms the bacterial cell wall

Structure

Peptidoglycan: a complex polymer forms the bacterial cell wall

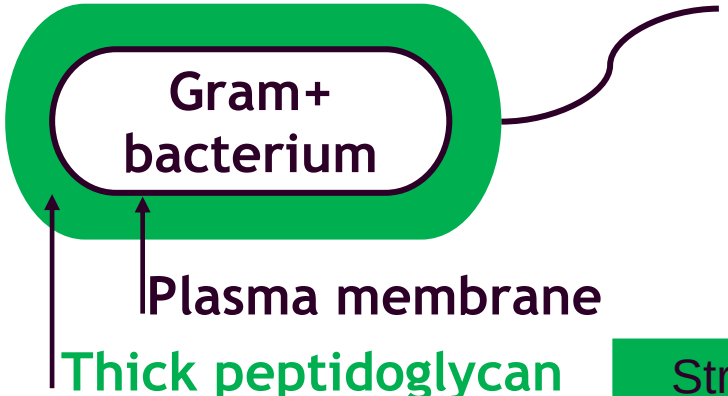


Structure

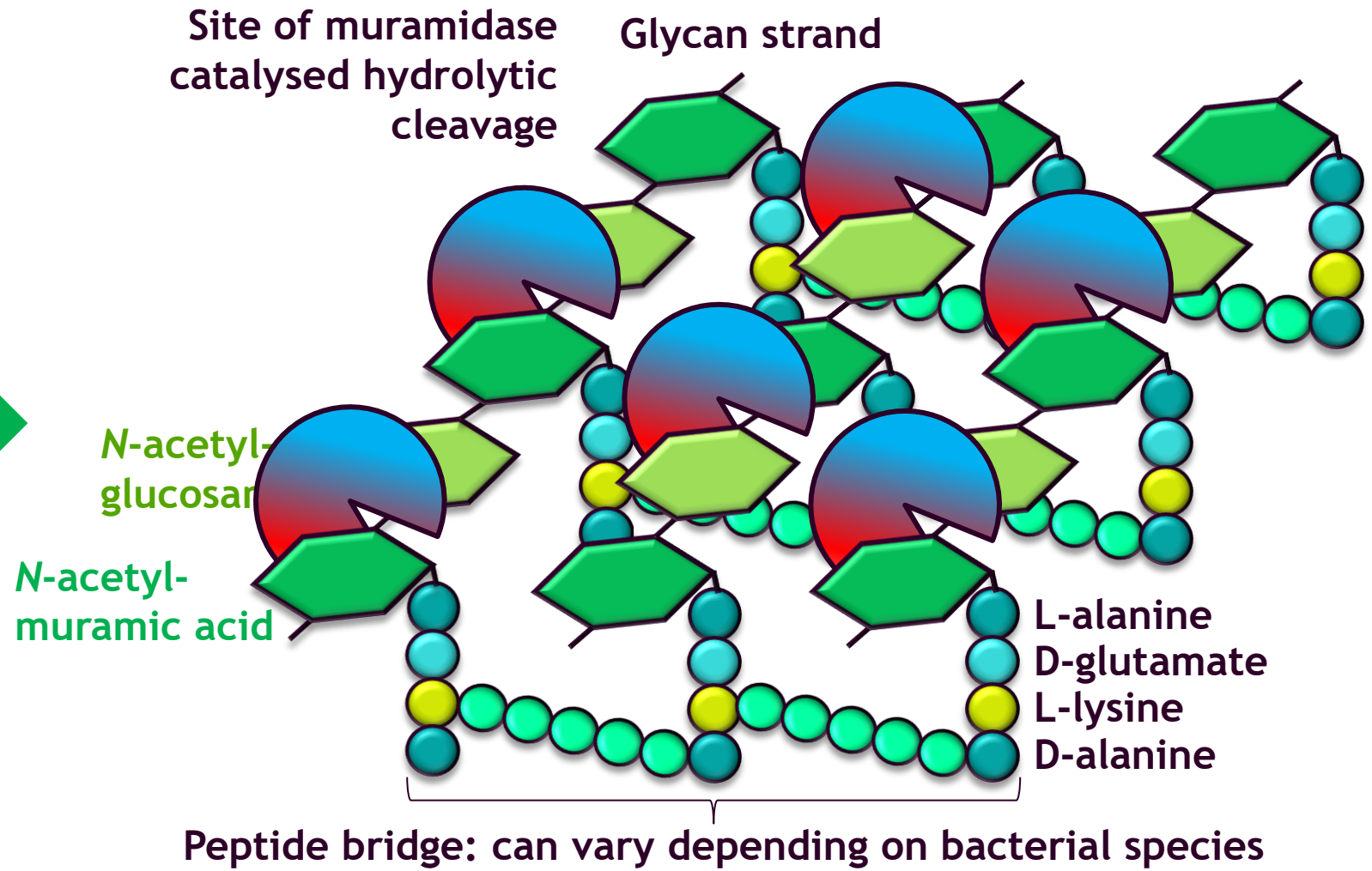


Solving the problem

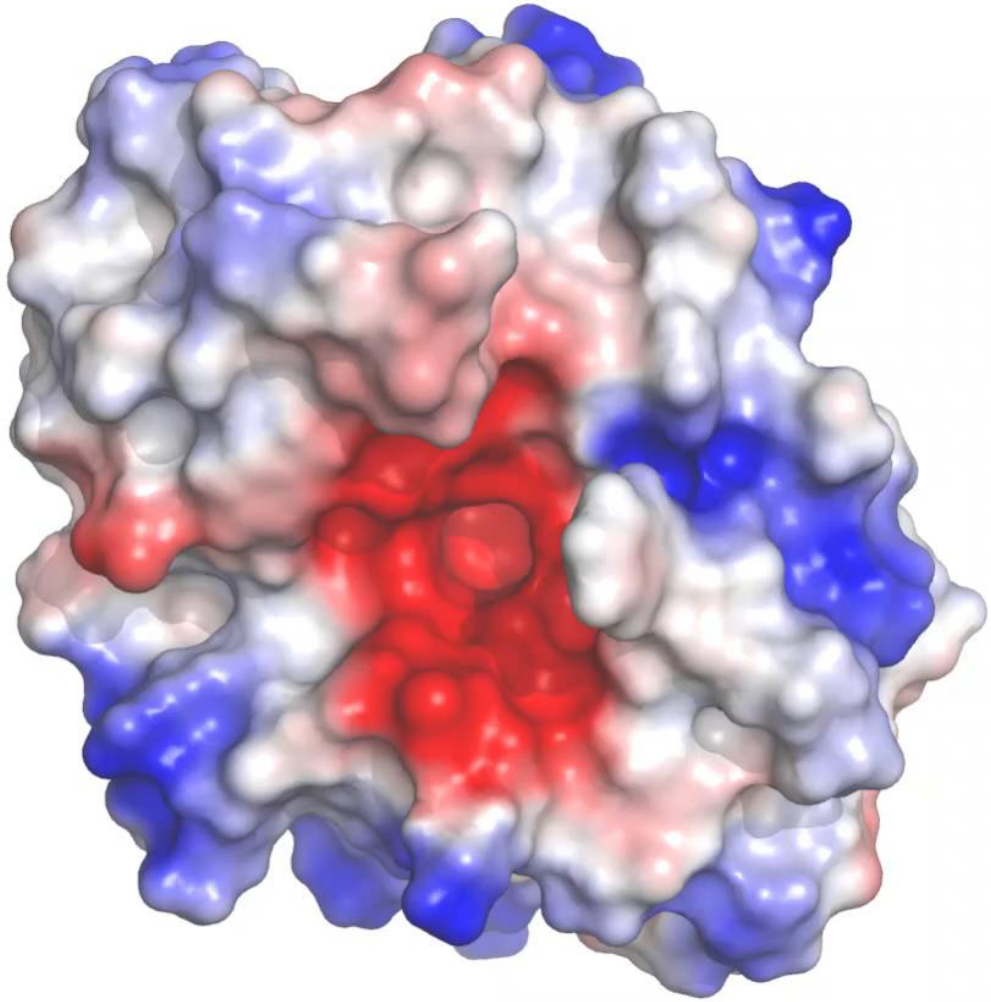
Using a novel microbial muramidase to cleave PGN



Structure



A novel microbial muramidase



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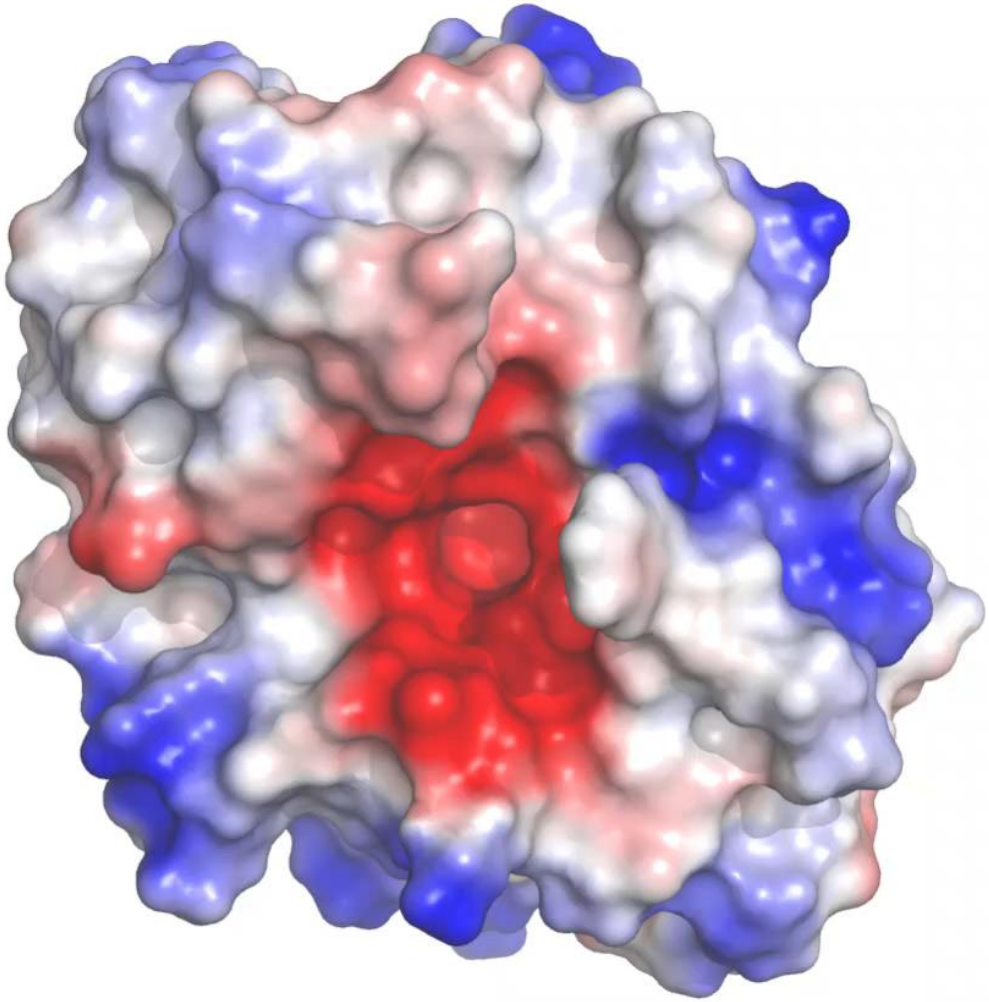
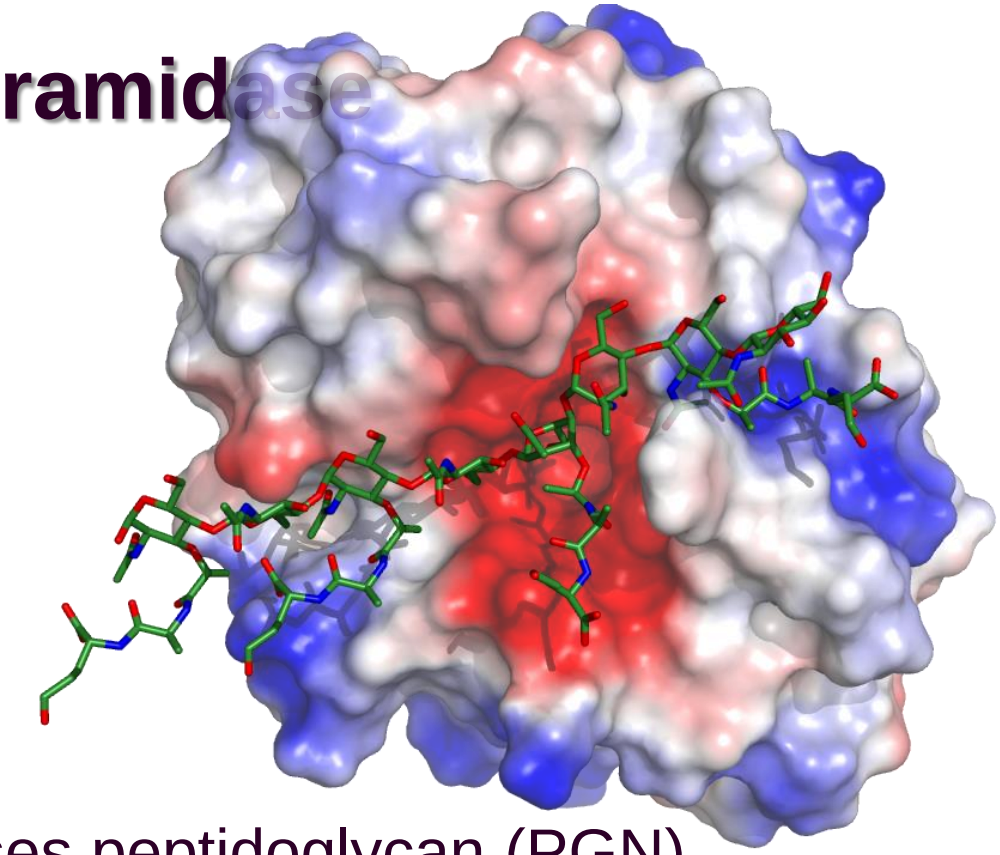
A novel microbial muramidase





- Hydrolyses peptidoglycan (PGN)
- β-1,4-N-acetylmuramidase activity
- Lack of apparent antimicrobial potency
- The only solution targeting dead and decomposing bacteria

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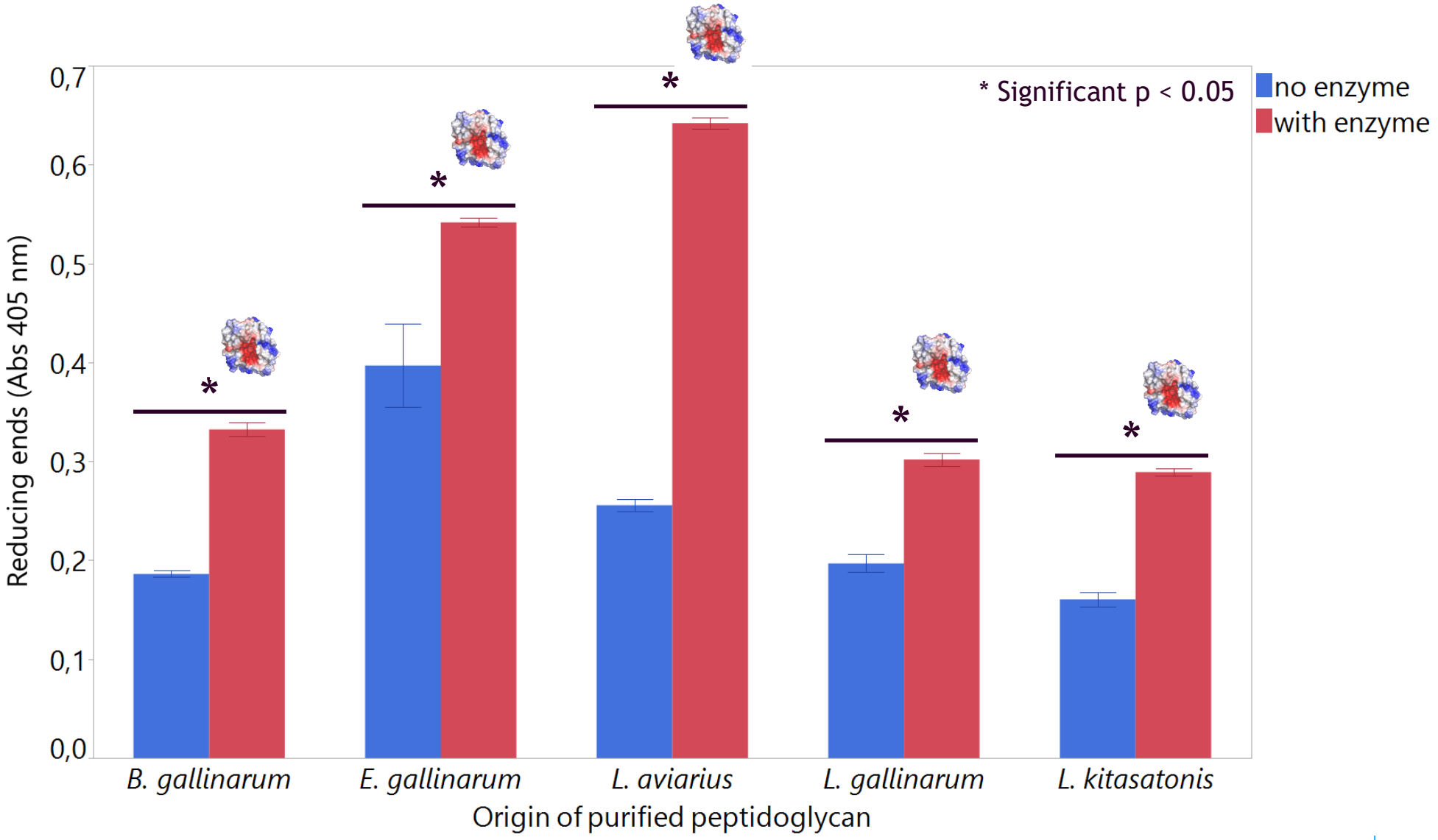
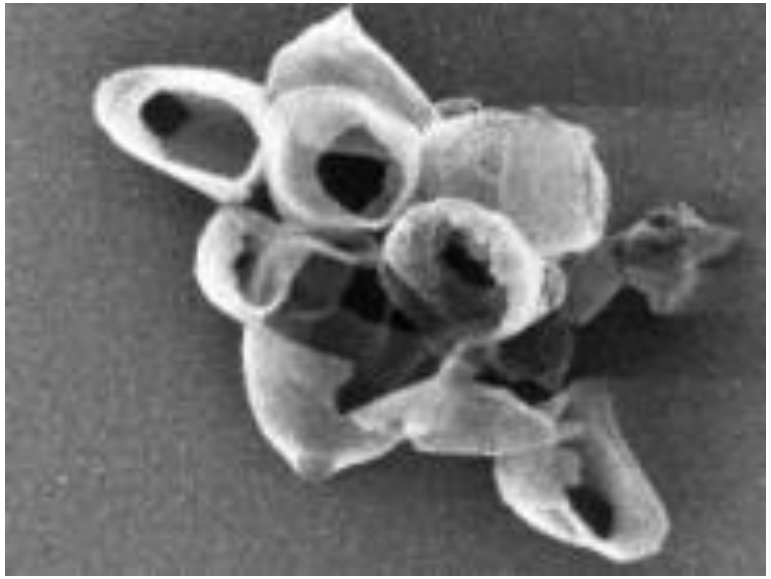
How the solution works

The novel microbial muramidase degrades peptidoglycan from relevant gut bacteria

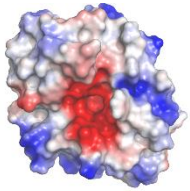


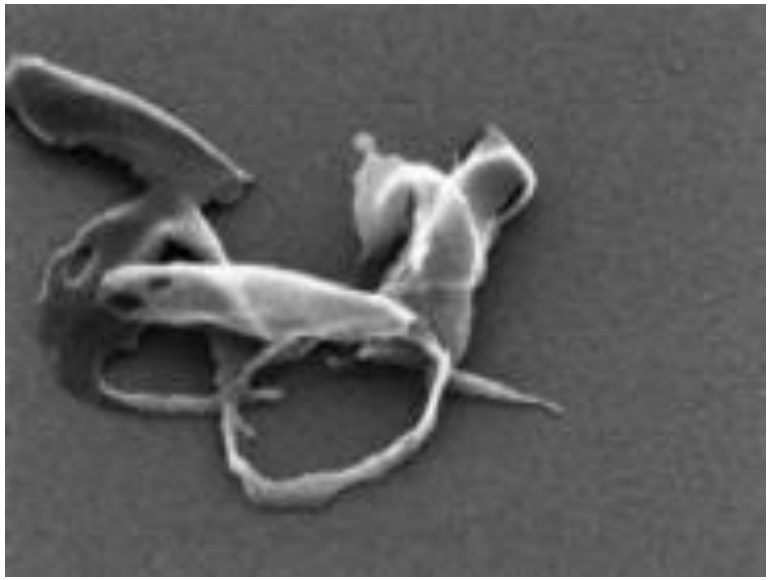
Microscopy of intact and hydrolyzed peptidoglycan

No enzyme



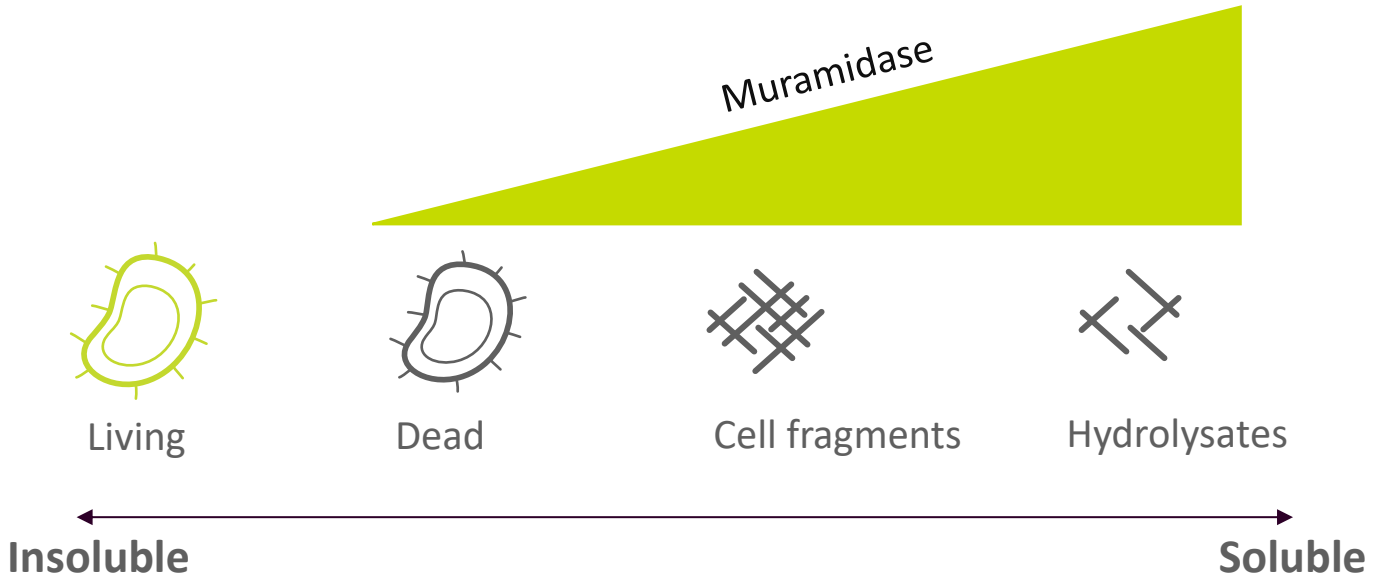
With enzyme



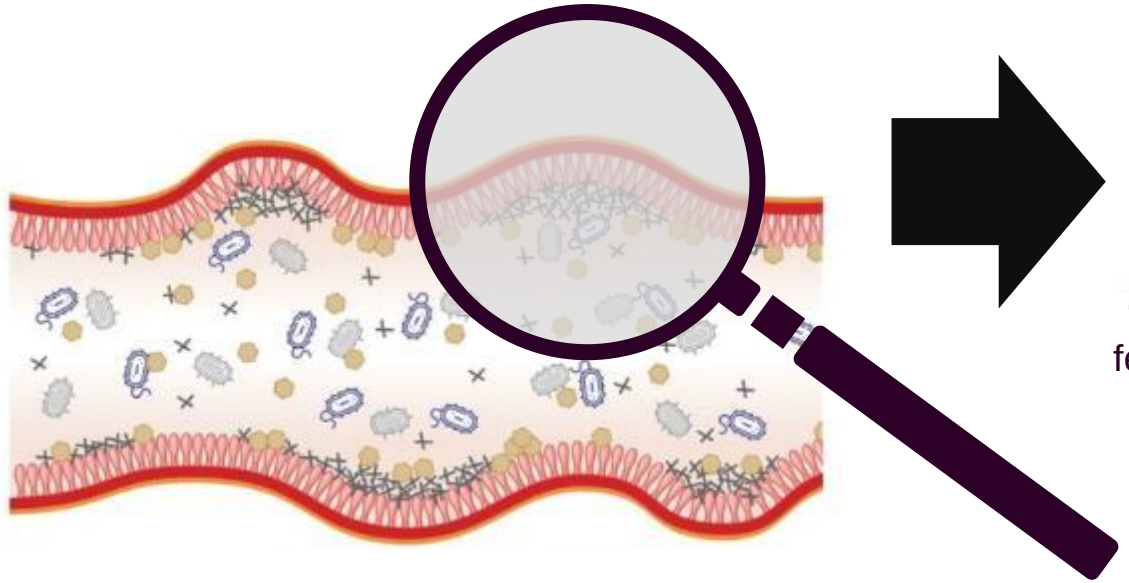


Enterococcus gallinarum

The novel microbial muramidase depolymerizes peptidoglycan into smaller fragments



Novel microbial muramidase: Impact on animal physiology



PGNs accumulate in the intestinal tract

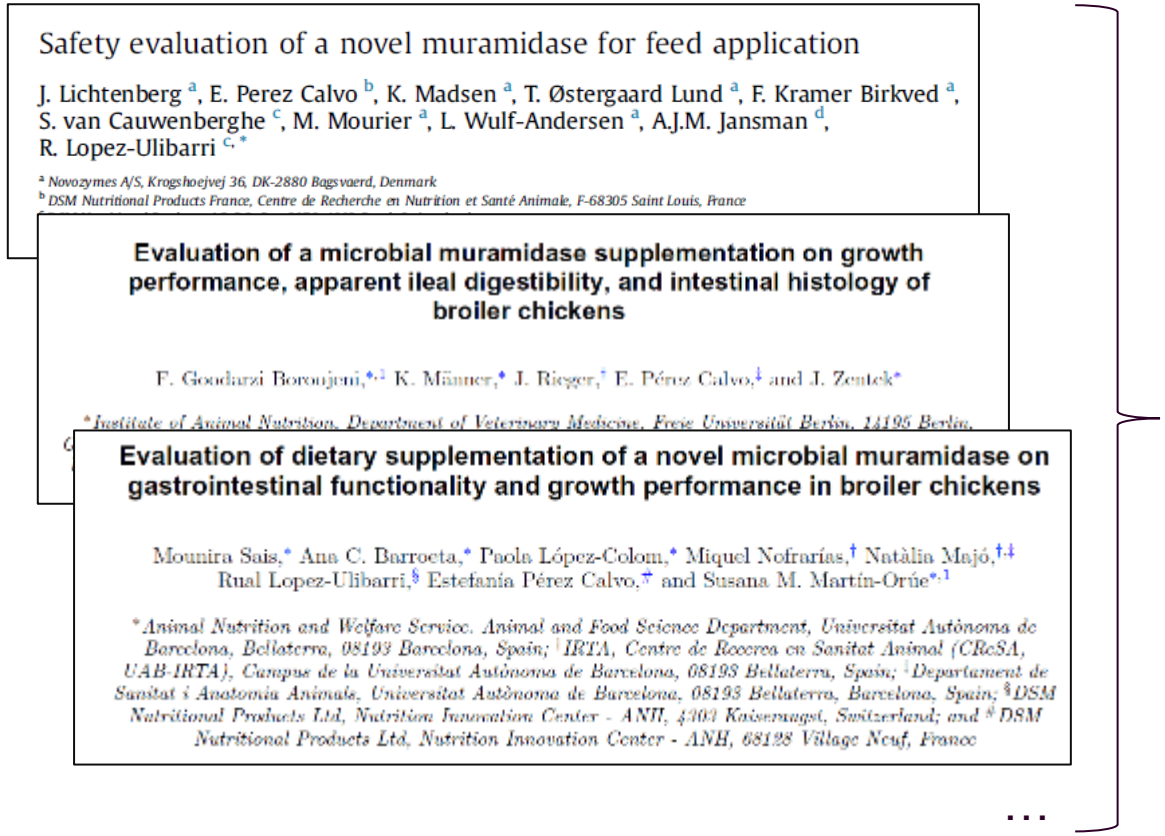
Novel microbial muramidase hydrolyzes PGNs

“Good” bacteria feeds on smaller PGN debris

Nutrients are now able to be absorbed

Performance improves from better nutrient absorption

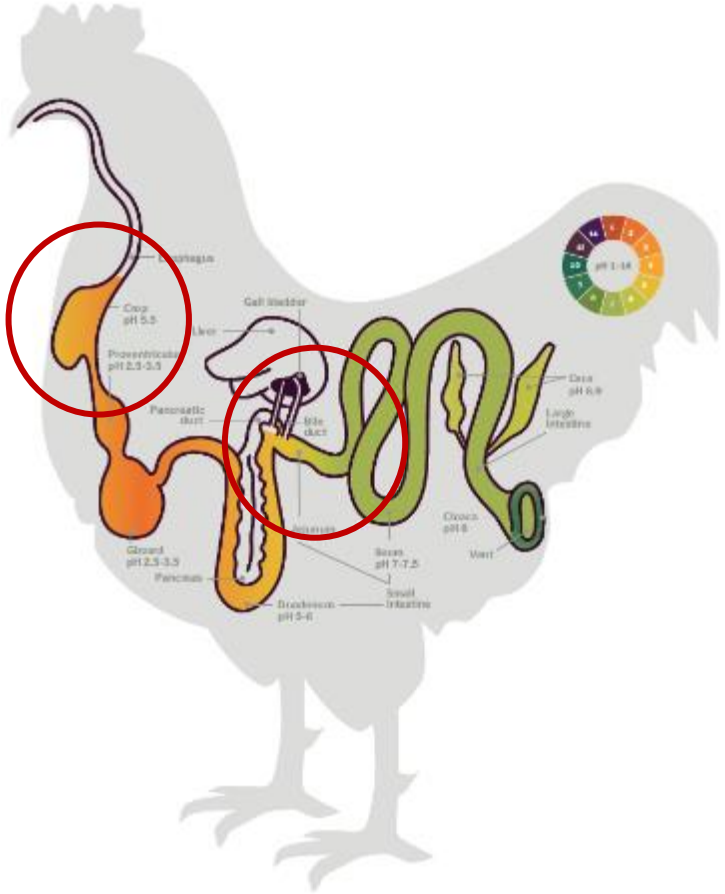
In vivo data of a novel microbial muramidase

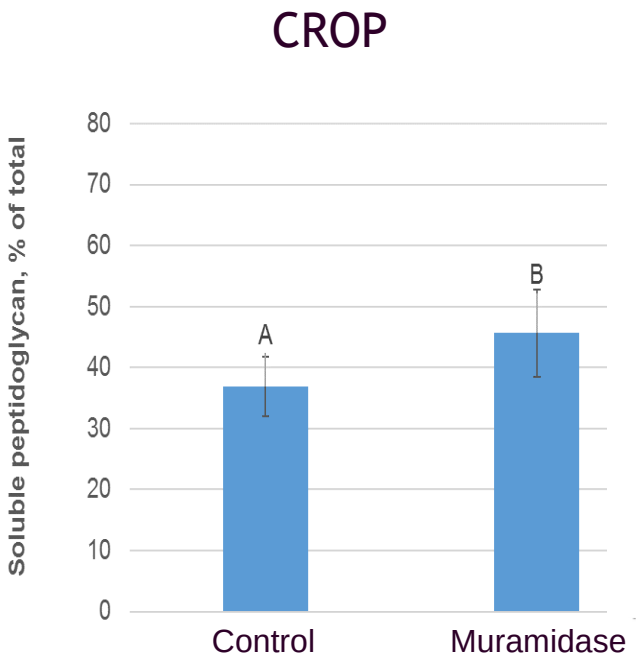


Muramidase supplementation in broiler chicken diet

- Delivers consistent effects in more than 50 *in vivo* studies performed globally.
- Increases body weight gain
- Improved utilization of feed (improved FCR)
- Increased digestibility of key nutrients
- A range of other beneficial effects
- Can save globally 9 million tons of CO2 equivalents.

Effects of the novel microbial muramidase can be measured throughout the GIT

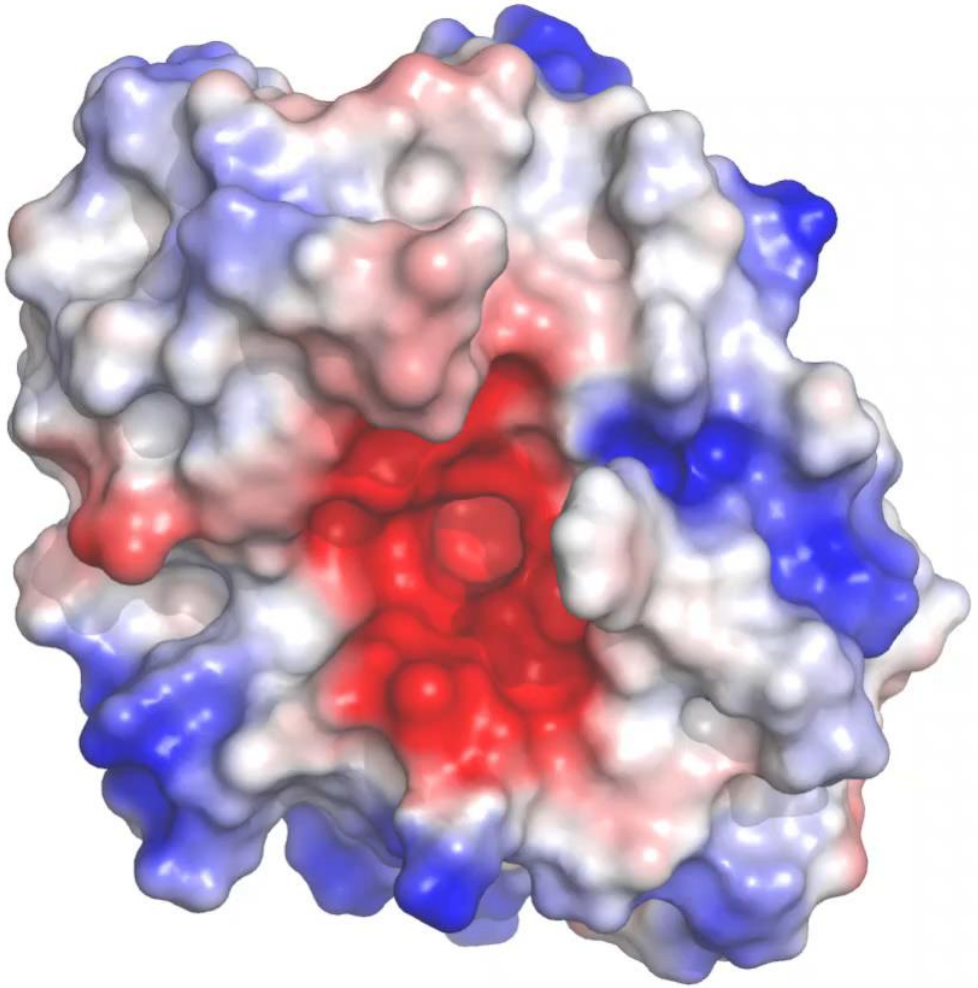




- Male Cobb 500, 35 days old
- Muramidase dose: 45 000 LSU(F)/kg
- Diet type: Corn-SBM- wheat (15%) + ionophore as coccidiostat

Source: ME-08/18VN E. Perez Calvo & R. Aureli

The novel microbial muramidase - first of its kind



- Muramidase enhances gut functionality by cleaning up bacterial debris from GIT
- Muramidase only degrades cell fragments, leaving live bacteria unaffected
- Muramidase catalyzed PGN degradation can be measured in vivo

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Thank you

Questions

Further informations about the novel microbial muramidase

Lack of anti-microbial potency confirmed *in vivo* and *in vitro*

In vitro
With 8X dose

No detection of antimicrobial potency in MIC assay

- MIC (Minimal Inhibitory Concentration) assay is the industry-standard measurement of antimicrobial potency
- 7 reference strains recommended by the European Food Safety Authority
- 30 field strains isolated from poultry

In vivo
With 10X dose

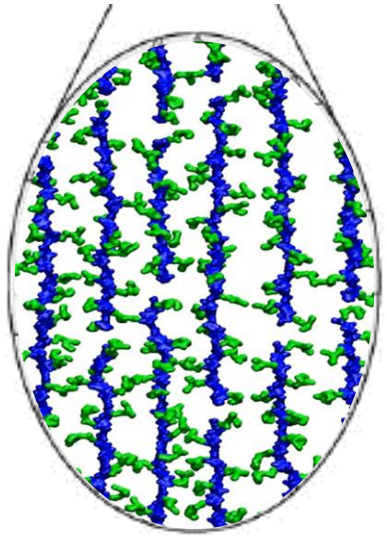
No significant reduction in caecal bacterial counts

- Total caecal aerobes and anaerobes (CFU/g)
- Enterobacteria, Coliforms, Lactobacilli (CFU/g)

Regul Toxicol Pharmacol. 2017 Oct;89:57-69, IPSF 2018 poster 271

Conformational selection: “Smart” muramidase distinguishes between peptidoglycan conformations

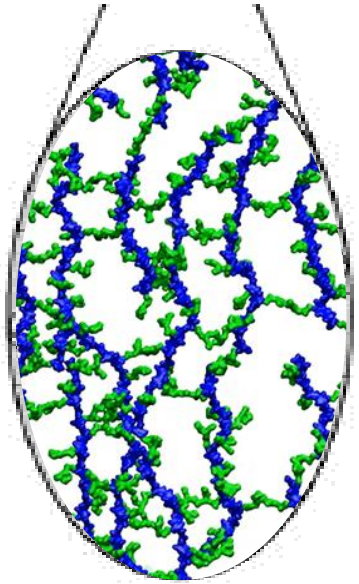
Live cells



Stretched due to pressure inside live cell

(Turgor pressure: up to 20 atm)

Dead cells



Relaxed due to lack of pressure from live cell

Trends Microbiol. 2010 Feb;18(2):59-66

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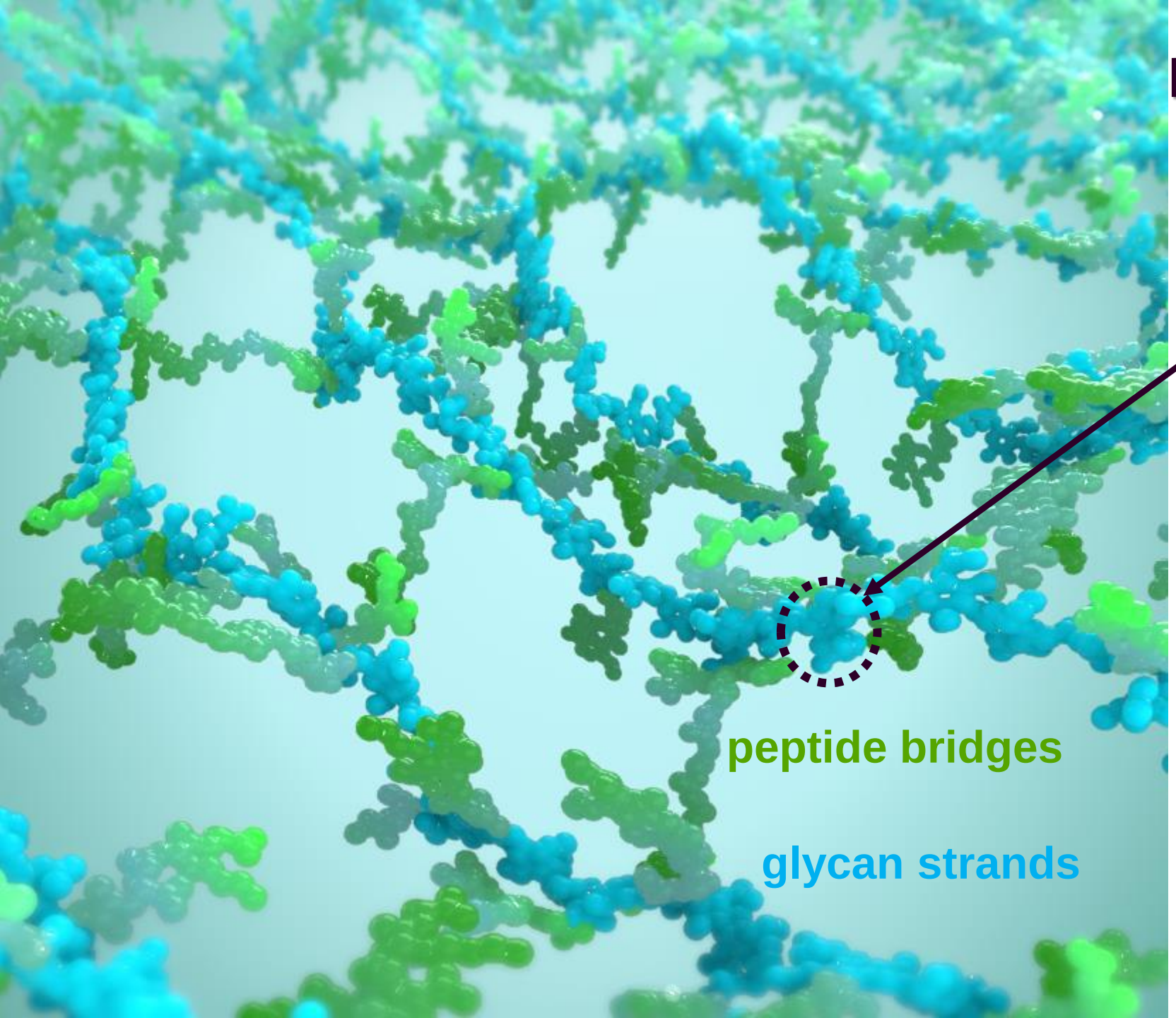

Monomer quantification

Chemical hydrolysis to single muramic acid sugars

5 M hydrochloric acid (pH<0)
for 24 hours at 100°C

Quantification of muramic acid

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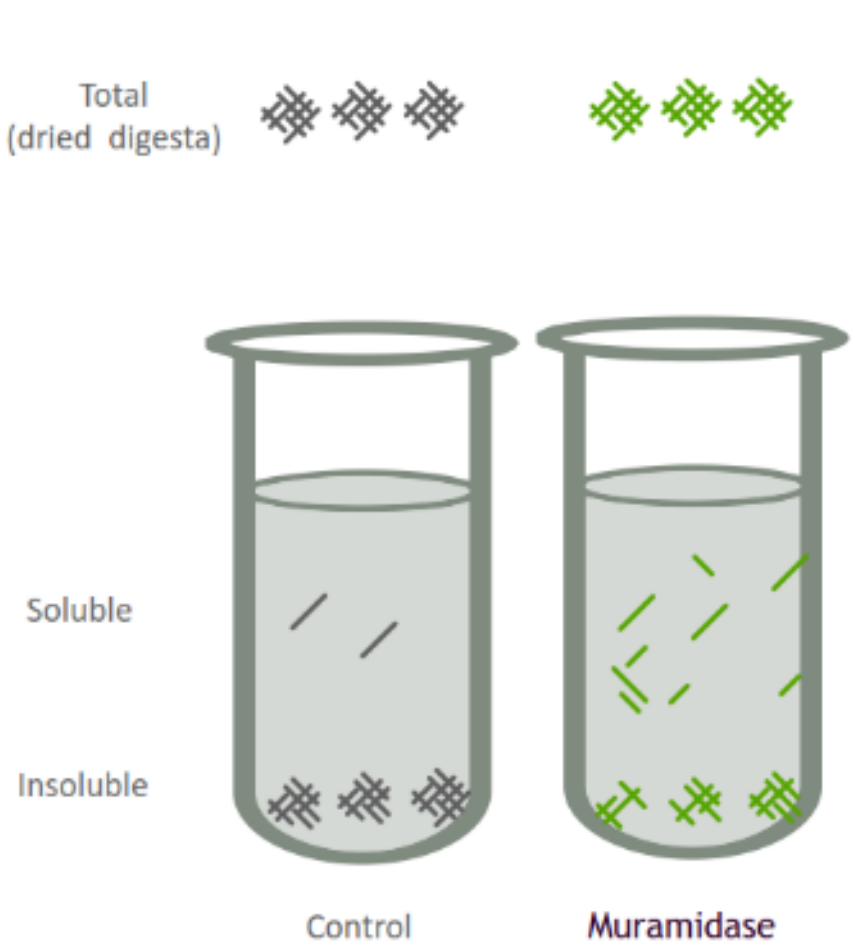
Novel method to quantify PGN degradation in digesta samples through muramic acid analysis

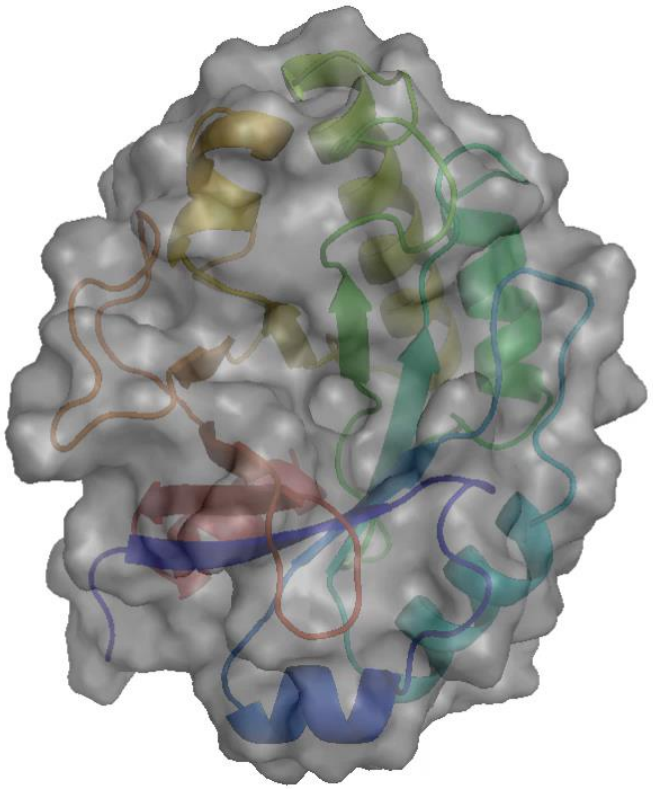


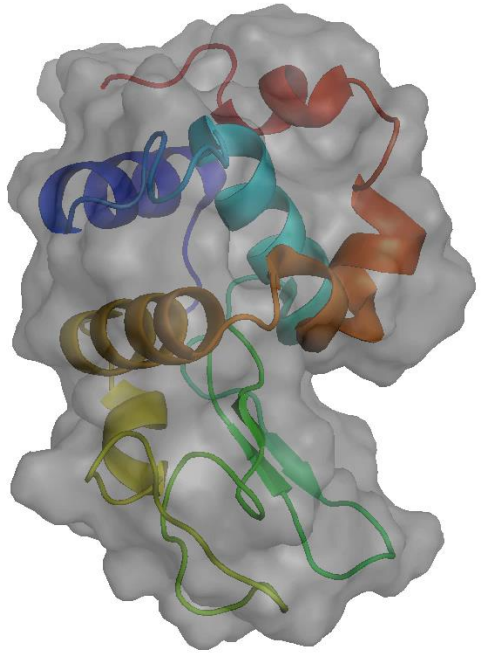
Acid hydrolysis & measurement of muramic acid

Acid hydrolysis & measurement of muramic acid

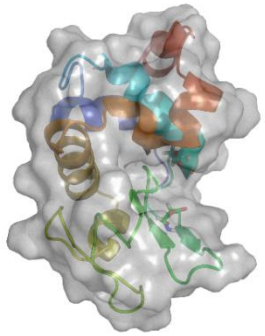
$$\textit{Ratio} = \frac{\text{Soluble muramic acid}}{\text{Total muramic acid}}$$

Muramidase vs hen egg white lysozyme





Chicken type lysozyme =antimicrobial



Hen egg white lysozyme and other higher animals

GH22

C-like lysozyme (c for chicken)

Hydrolysis of 1→4 beta linkage

Found in milk, saliva, mucus, tears, egg-white

First enzyme, structure was solved in 1960ies

Classical Koshland retaining mechanism containing covalent glycosyl intermediate

Side activity on chitin (also 1→4 beta linkage)

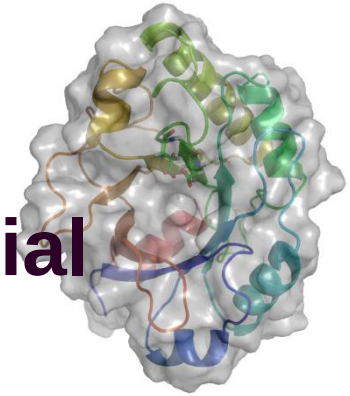
Glu35=cat residue, Asp52 catalytic nucleophile in large cleft

127 aa, 4 disulphide bonds, 5 helical regions (40% of aa), five regions of beta sheet with rc and beta turns

Structure shard with GH19 (chitinases), GH23(lysozymes (goose)), GH124 (cellulases), GH134 (mannanases)

~130 aa

Novel microbial mura-Midase =not antimicrobial



Fungal muramidase

GH25

“Chalaropsis”-like lysozyme

Hydrolysis of 1→4 beta linkage

β-1,4-N-acetylmuramidase activities

Likely retaining

Mechanism=neighboring group participation

Structure unrelated to GH22 etc.

TIM-barrel (eight-stranded beta barrel flanked by 6 (normally 8) alpha helices

Long groove in C-terminal face, culminating in deep whole of highly neg electrostatic potential = cat site

DIE motif (D and E cat residues, around pos 100)

~200 aa

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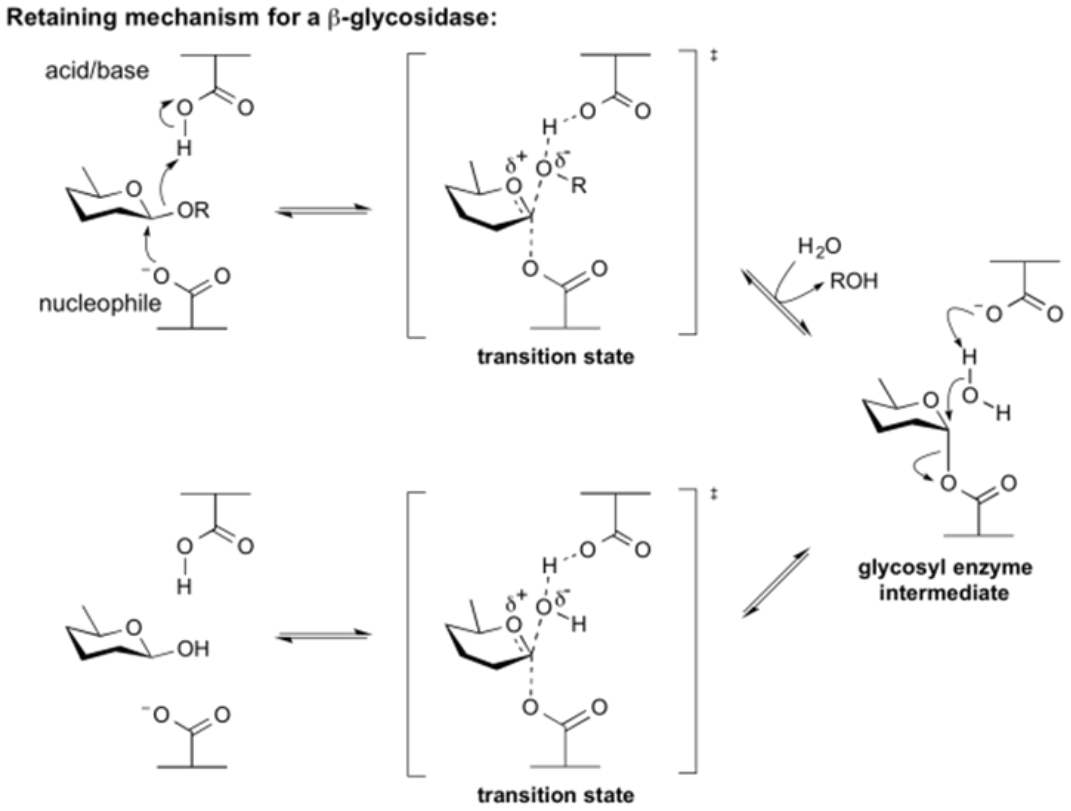
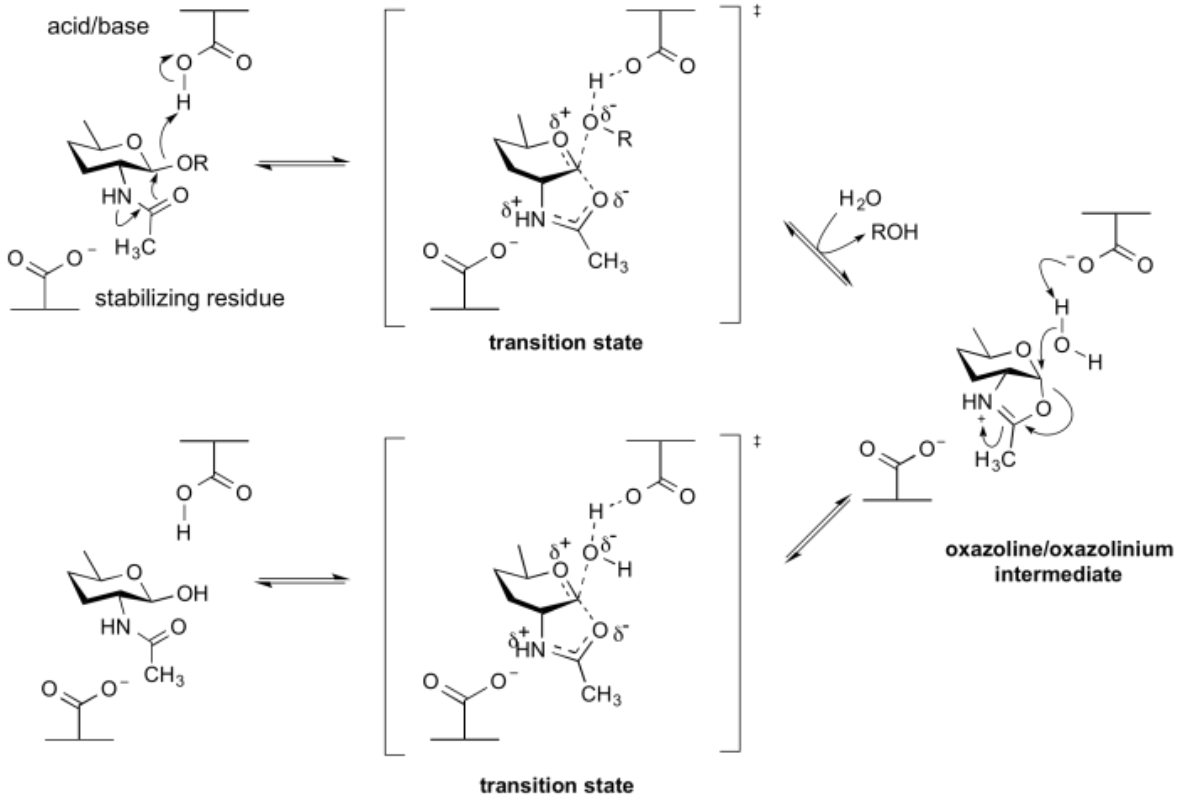
Mechanism



HEWL-koshland mechanism



Novel microbial muramidase: neighbouring group participation