Utrecht University

Veterinary Medicine

26-10-2020

REPROGRAMMING THE INNATE IMMUNE SYSTEM AS AN ALTERNATIVE

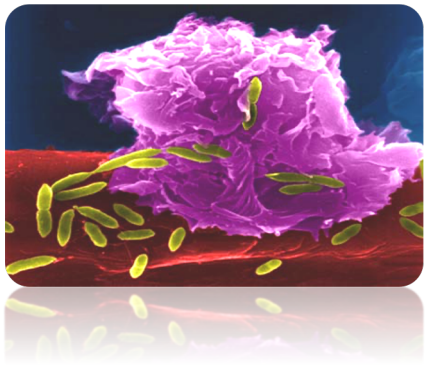
Henk P. Haagsman, Maaike R. Scheenstra, Edwin J. A. Veldhuizen & Albert van Dijk

Department of Infectious Diseases & Immunology

Innate Host Defense

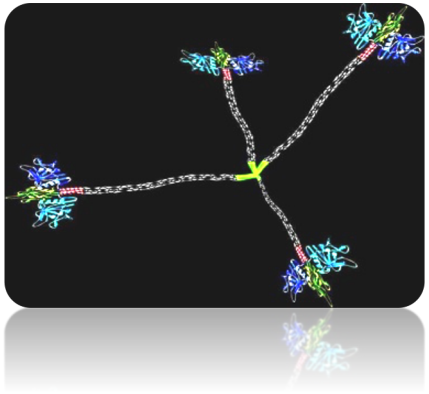
- Present in all organisms
- Limited repertoire of molecules
- Rapid
- Broad specificity
- Ancient

The first line of defense against infections



Cellular defenses

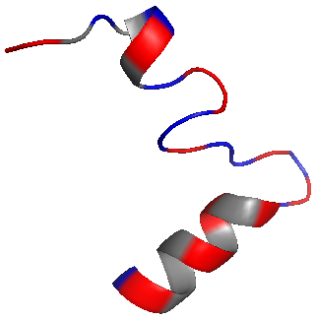
Neutrophils (heterophils)
Macrophages, NK-, IL-cells



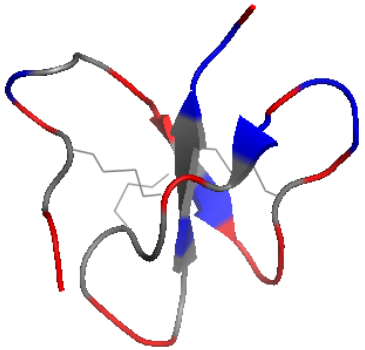
Effector molecules

Enzymes, Host defense peptides, Collectins

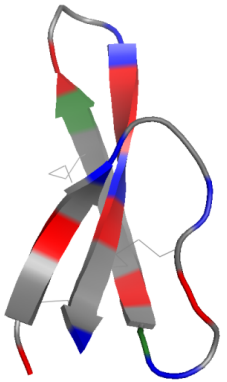
Examples of vertebrate host defense peptides



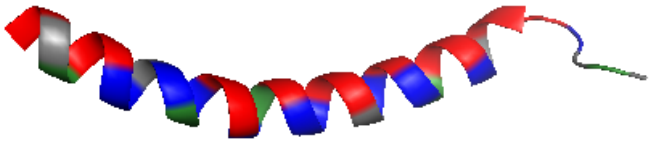
CATH-2



HBD-2



HD-5



LL-37

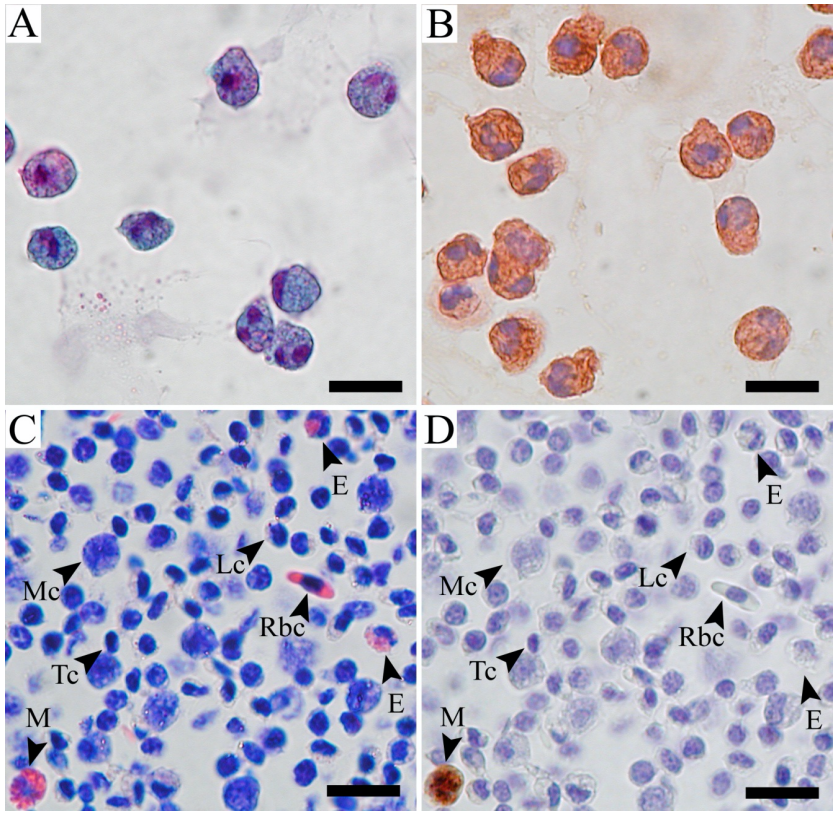
Magainin-2

Amino acid side chains: red, hydrophobic; blue, basic; green, acidic

CATH-2 is produced by chicken heterophils

Heterophils

Mononuclear cells

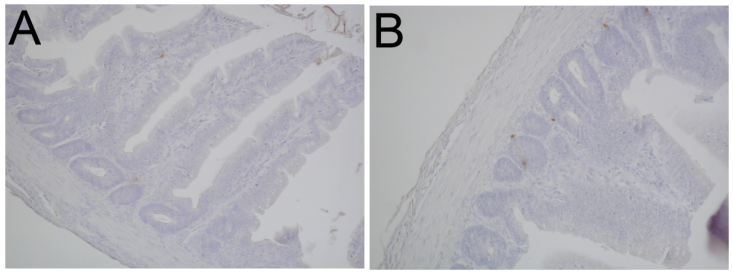


Giemsa Anti-CATH-2

Van Dijk et al., 2009a

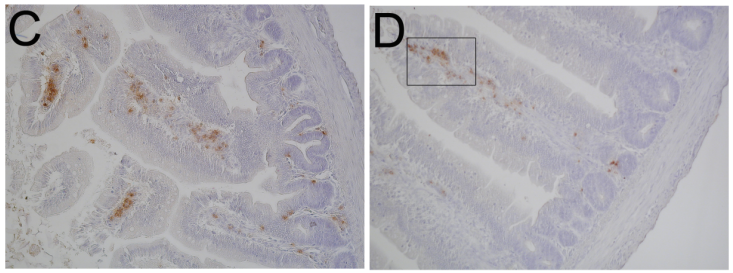
Salmonella enteritidis challenge of chickens results in recruitment of CATH-2 containing heterophils

Control
8h p.i.



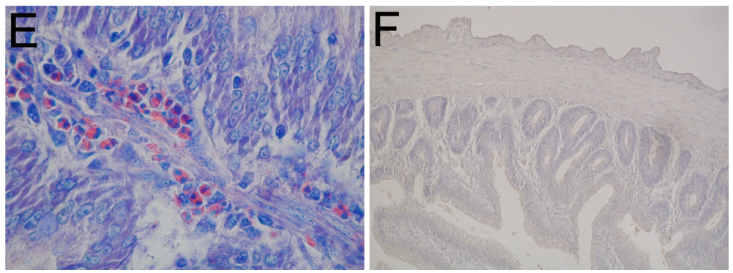
Control
48h p.i.

S. enteritidis
8h p.i.



S. enteritidis
48h p.i.

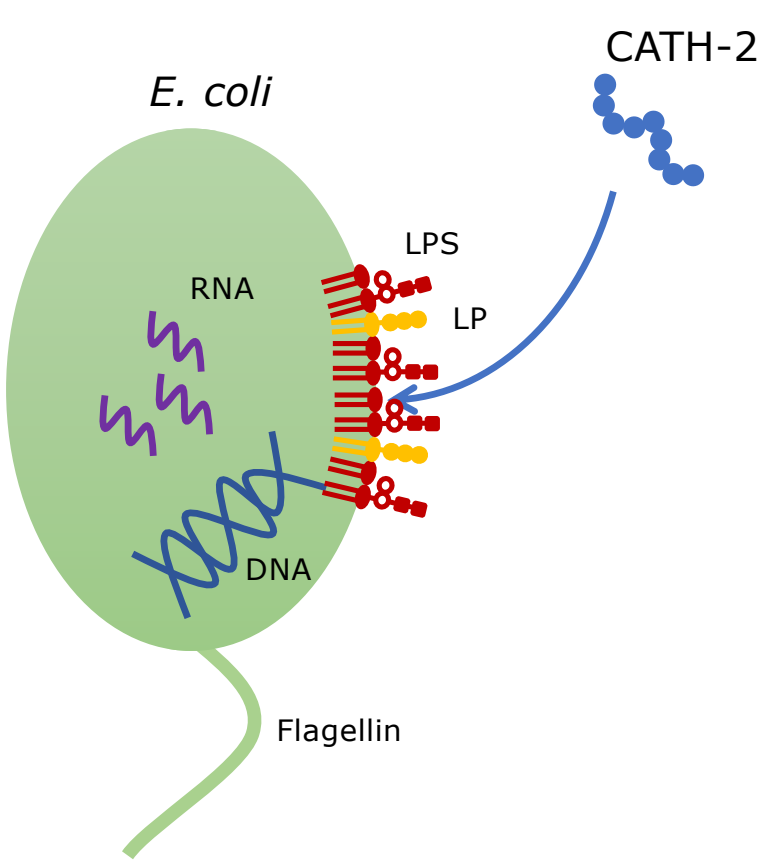
S. enteritidis
48h p.i.
(Giemsa)

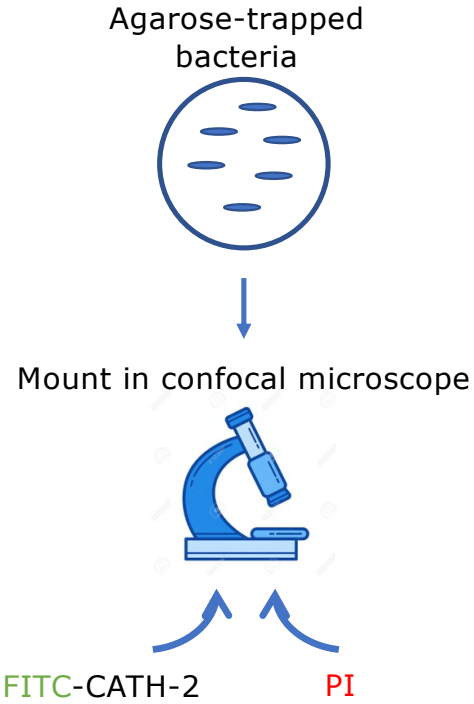


S. enteritidis
48h p.i.
(preimmune)

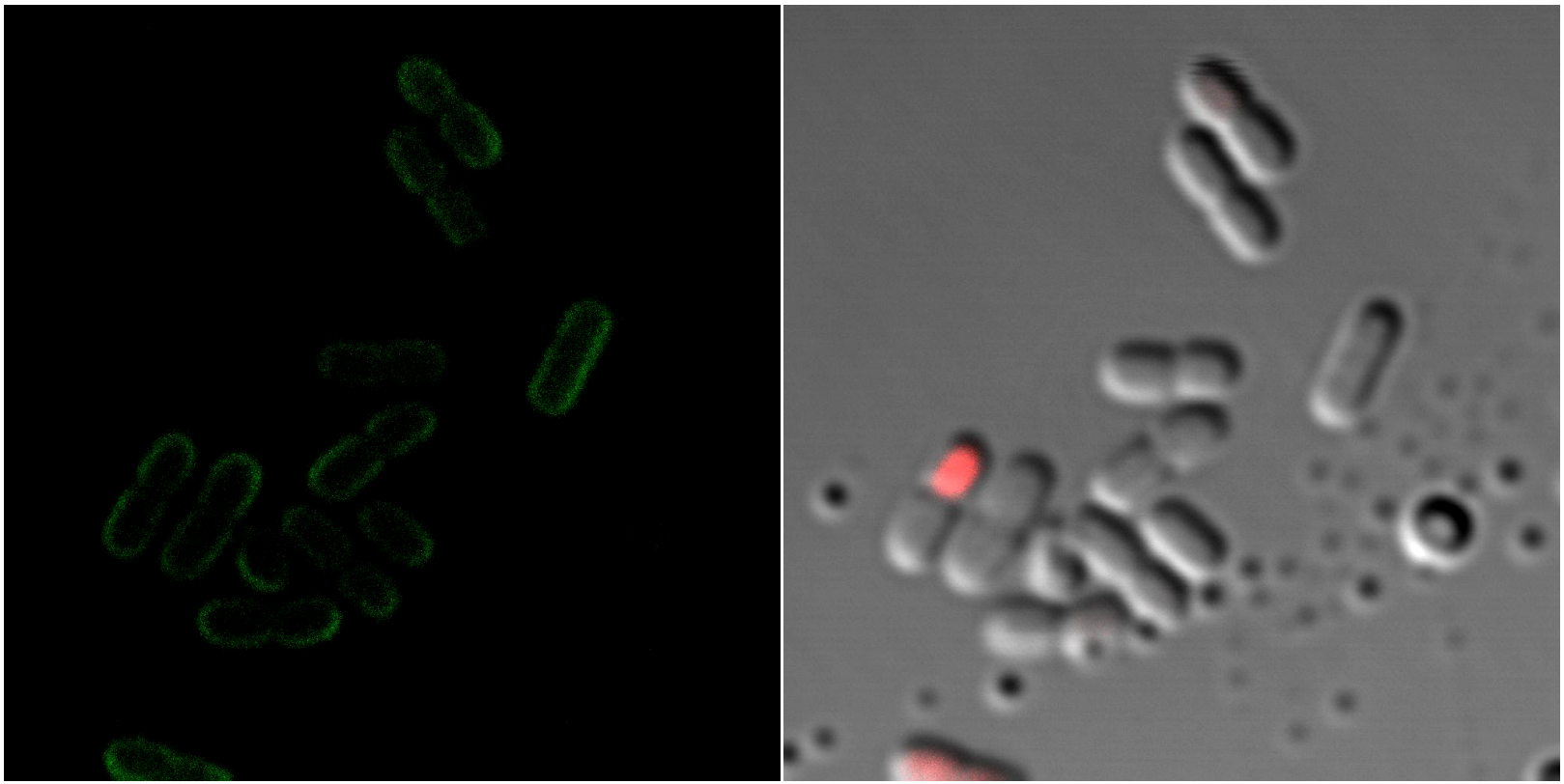
Van Dijk et al., 2009a

Visualization of CATH-2 / *E. coli* interactions



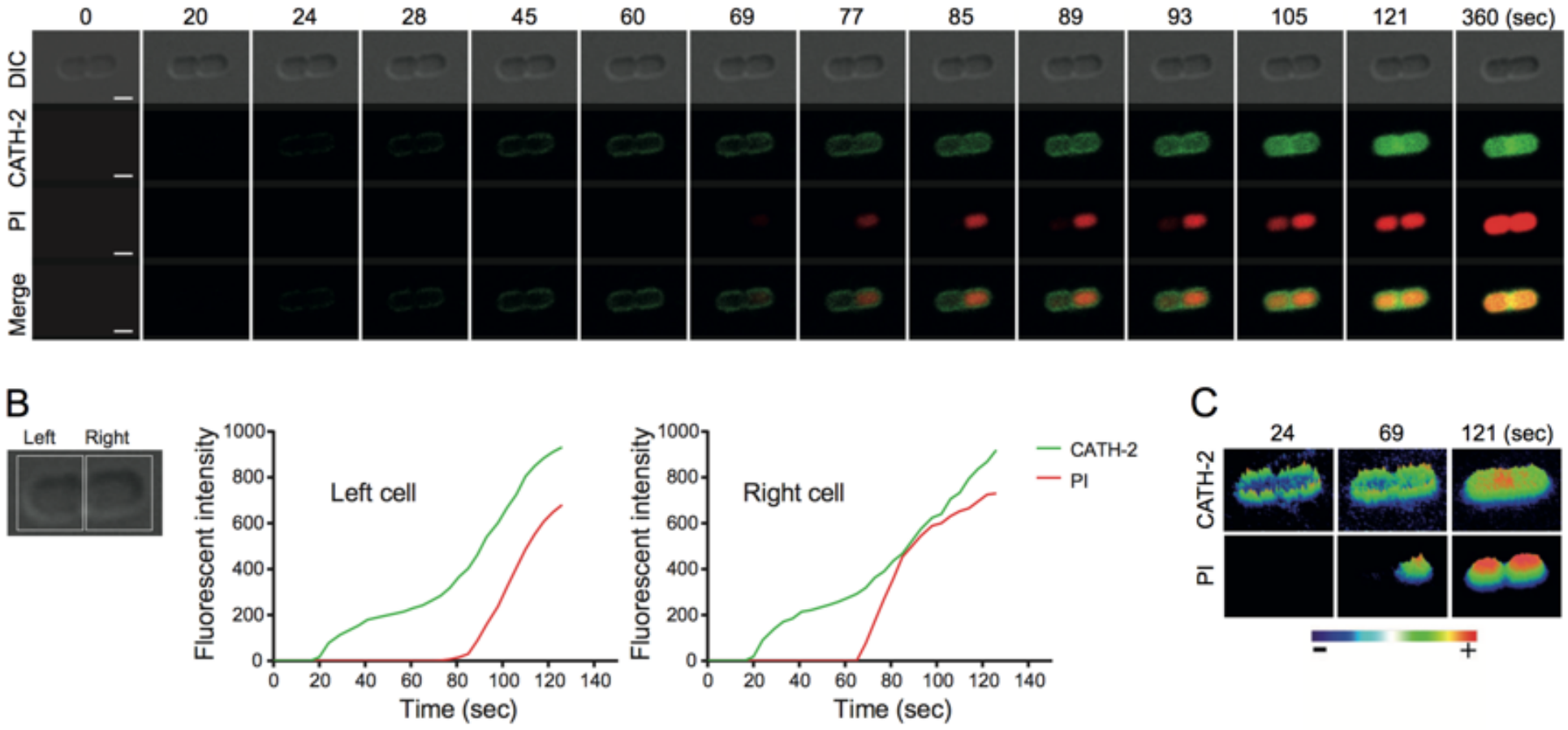


Live imaging of CATH-2-mediated *E. coli* killing



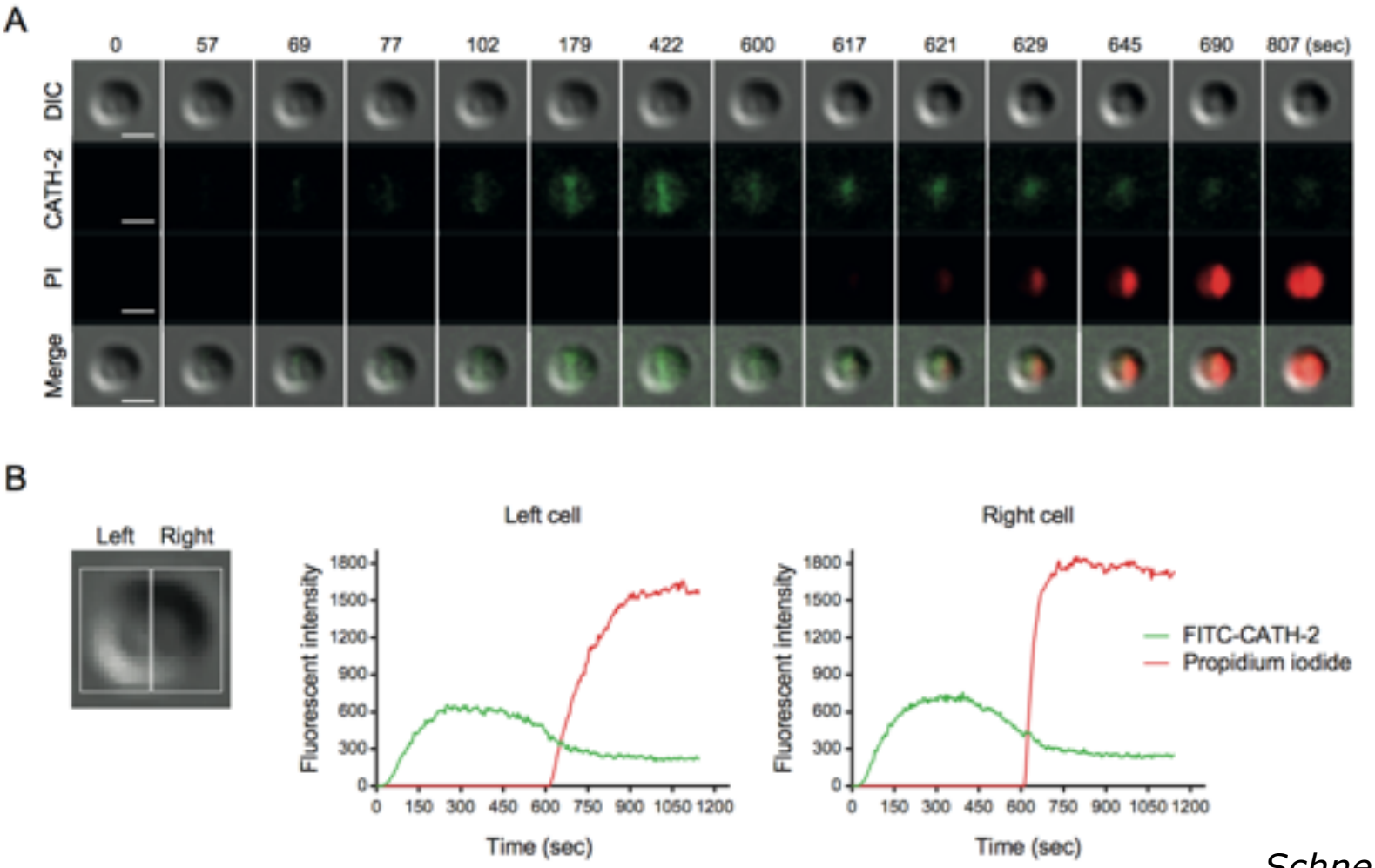
Schneider et al., 2016

Fast membrane binding and permeabilization of *E. coli* by CATH-2



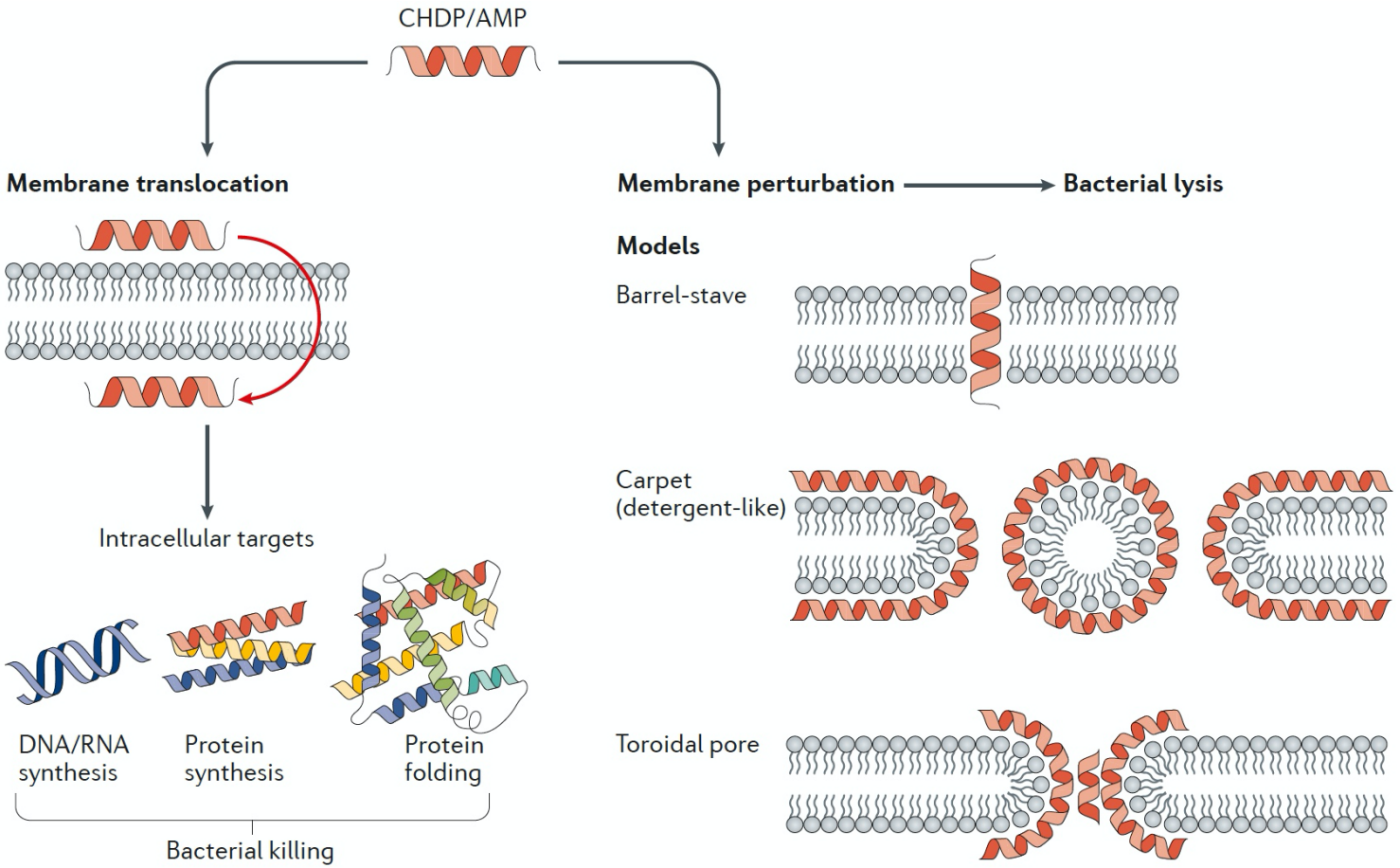
Schneider et al., 2016

Fast membrane binding and permeabilization of *S. aureus* by CATH-2



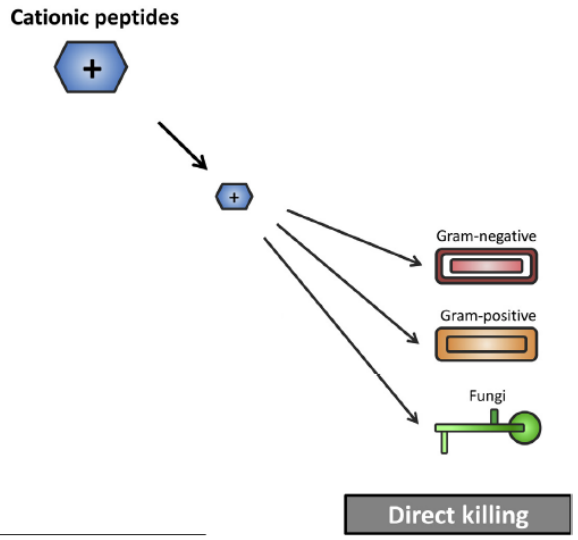
Schneider et al., 2017

Antibacterial mechanisms of Cationic Host Defense Peptides



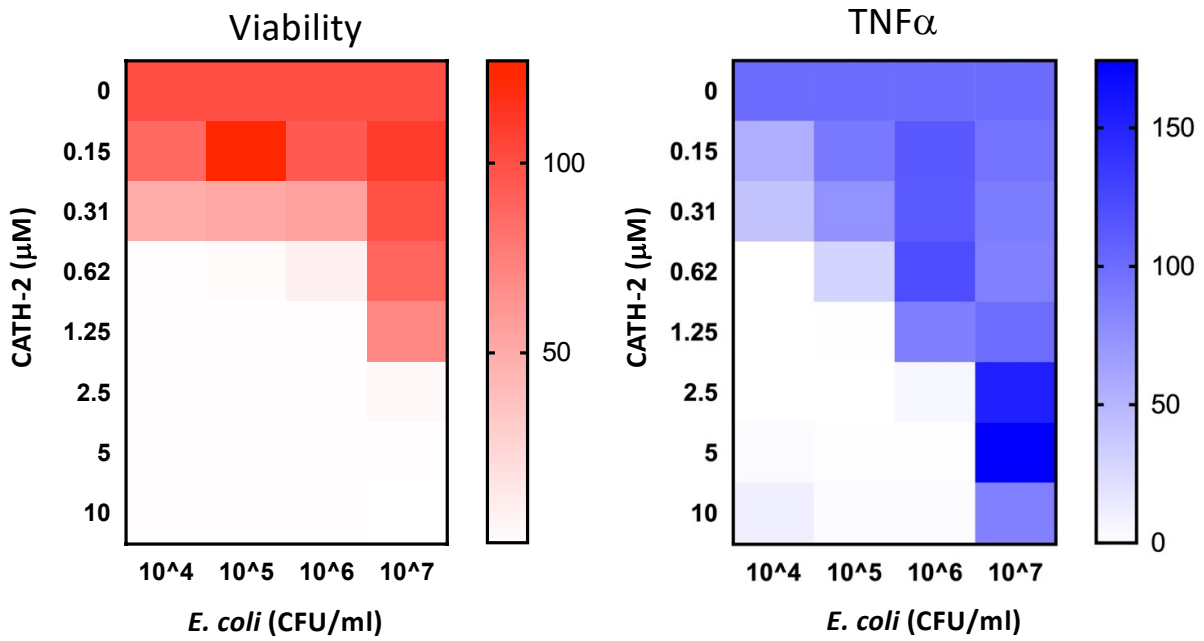
Mookherjee et al. Nat. Rev. Drug Discov. 2020

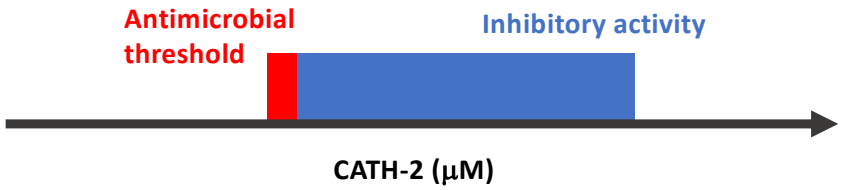
Functions host defense peptides



Cuperus et al. 2013

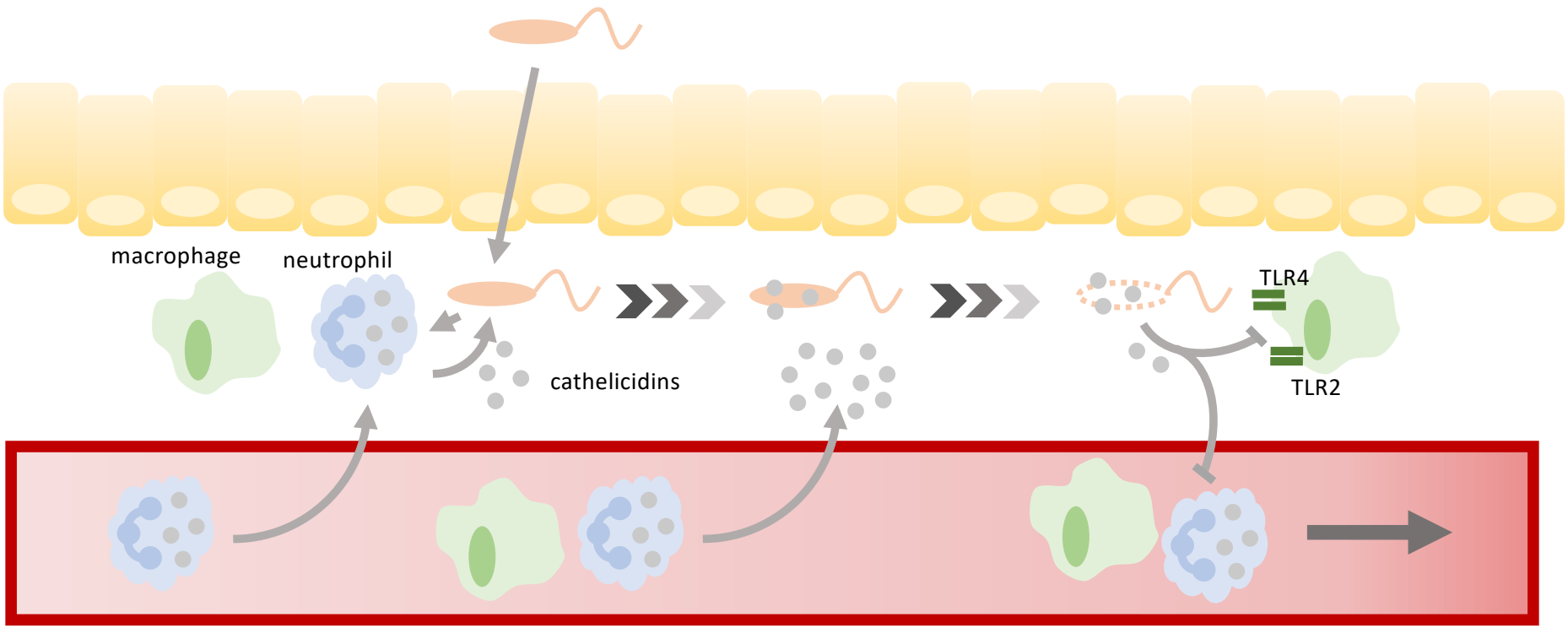
Antimicrobial activity vs. inhibition of macrophage activation





Coorens et al., 2017

Cathelicidin-mediated “silent killing”



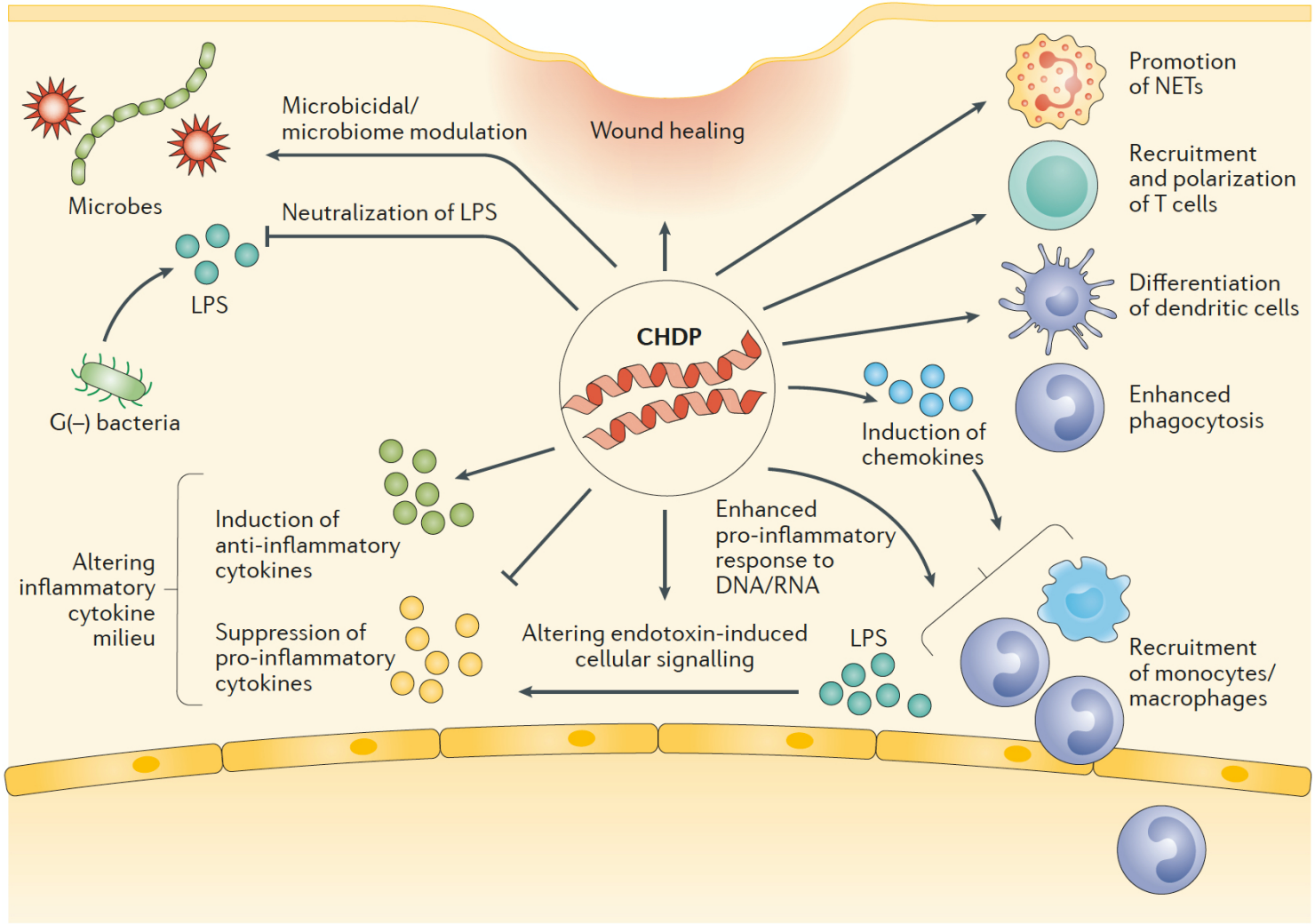
Host Defense Peptides / Antimicrobial Peptides as anti-infectives

Strategies:

- Administered HDP-based peptides as alternatives to antibiotics
- Gut: feed additives that stimulate endogenous production of HDPs

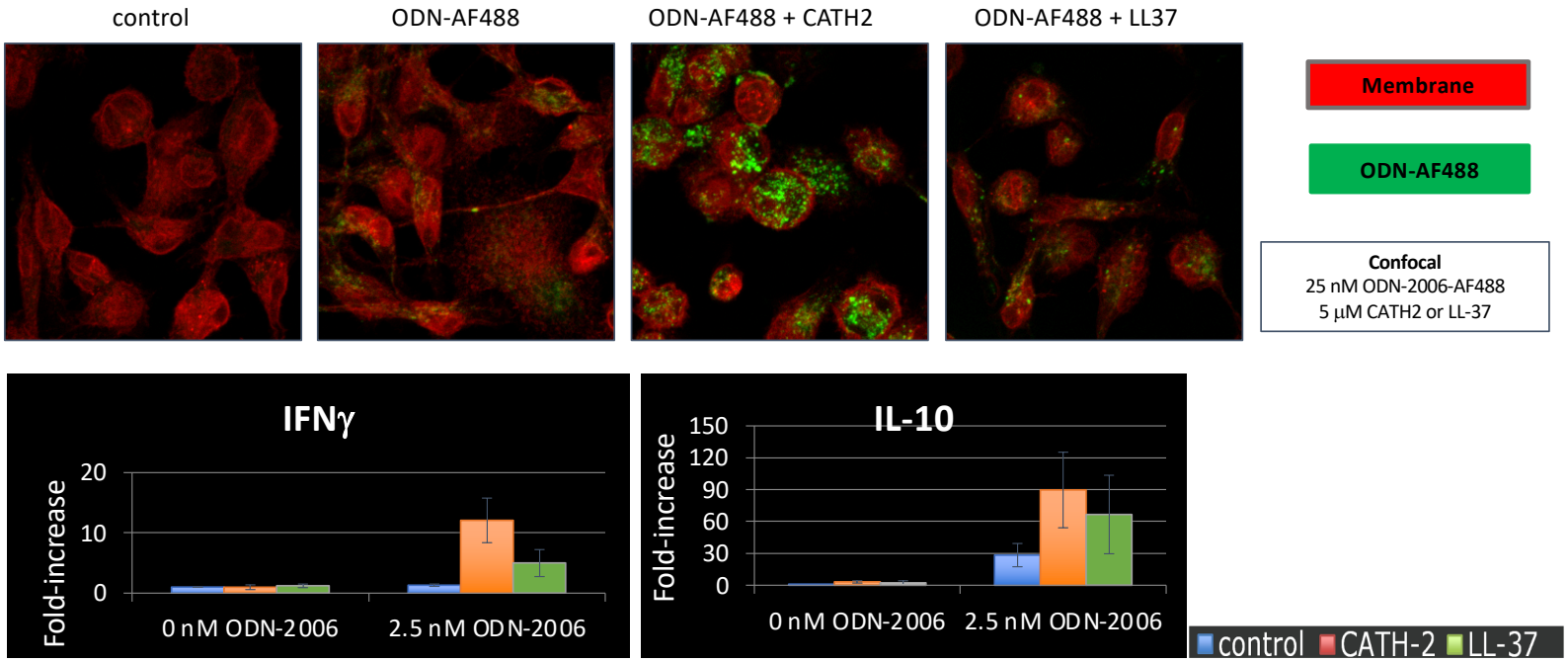
  (presentation of dr. Guolong Zhang)

Immunomodulation by Cationic Host Defense Peptides



Mookherjee et al. Nat. Rev. Drug Discov. 2020

CATH-2 enhances DNA uptake by macrophages



Enhancement of TLR9 (mammals) or TLR21 (birds) activation by CpG in vaccinations

Coorens et al. 2015

PRODUCT INFO

English | Español

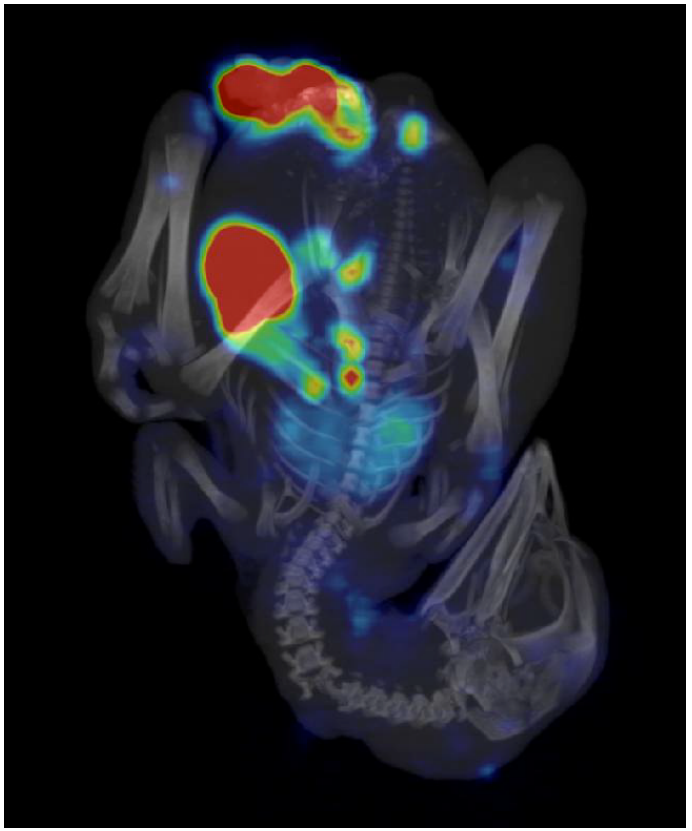
Zelnate® DNA Immunostimulant: a new chapter in BRD management

Bovine Respiratory Disease (BRD) is a complex disease with many contributing factors—including stress that compromises the immune system. Even though there are many antimicrobial options for BRD, veterinarians and producers continue to look for new technology to treat BRD. Zelnate DNA Immunostimulant is a novel, non-antibiotic technology that enhances the animal's natural defenses against BRD. Learn more about BRD

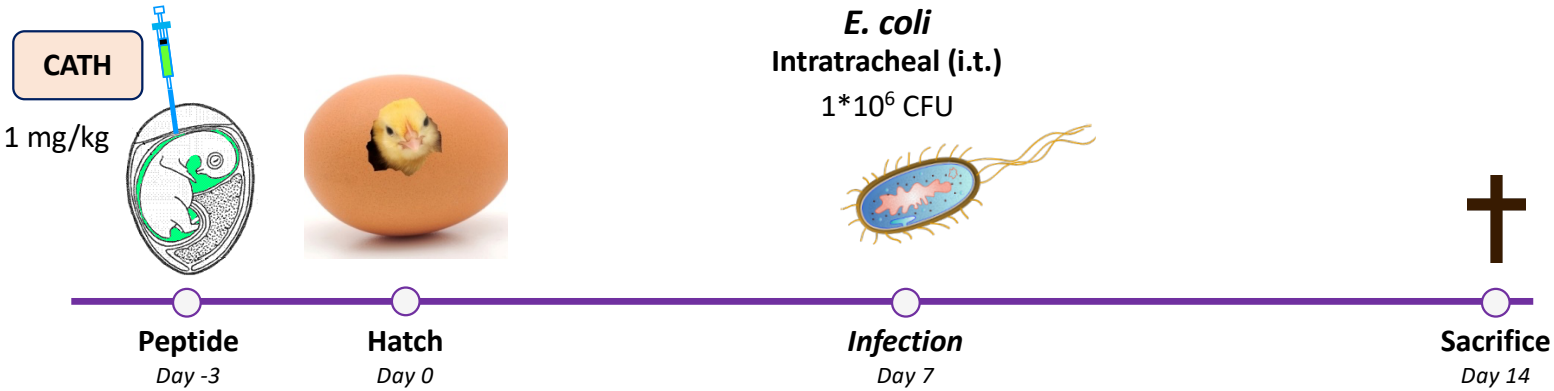
Zelnate is the first licensed immunostimulant that aids in the treatment of BRD associated with *Mannheimia haemolytica.*

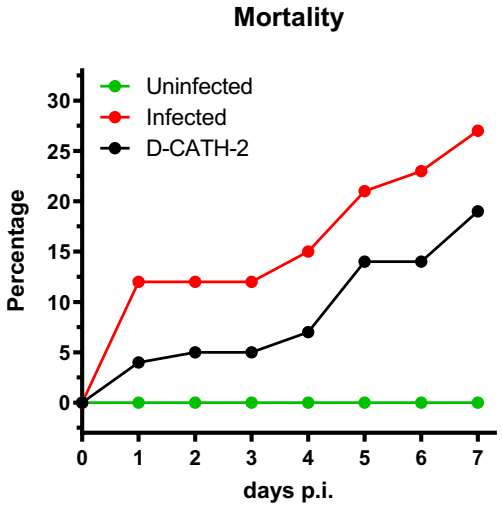
Prophylactic potency of HDPs and derivatives

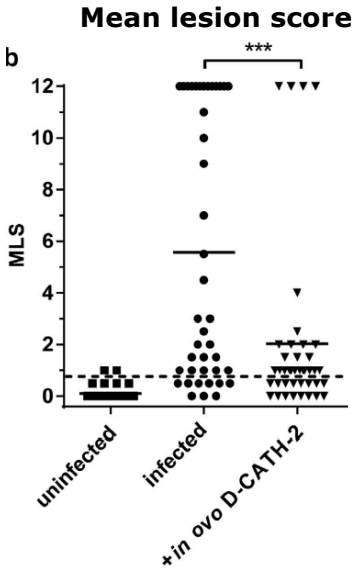
In ovo administration (chicken embryos)

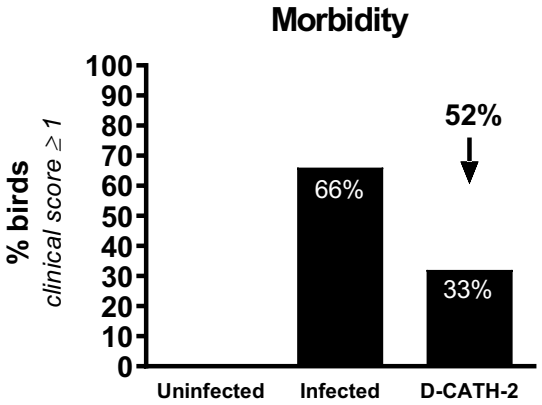


In ovo D-CATH-2 administration protects from colibacillosis



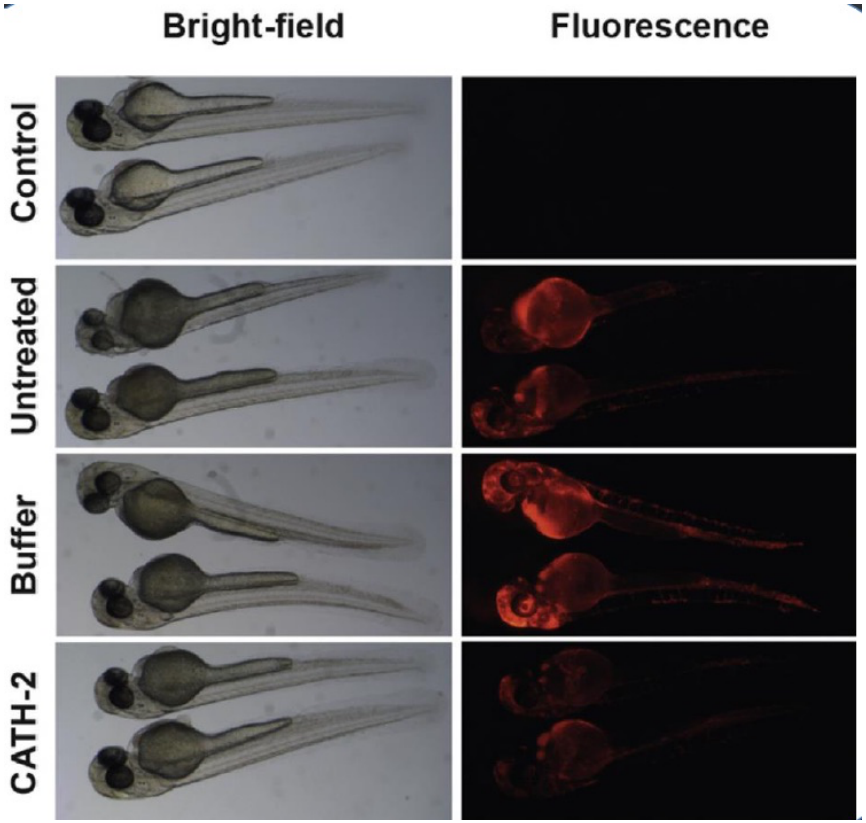






Cuperus, et al., 2016

Efficacy of D-CATH-2 in a zebrafish infection model



Zebrafish embryos

Yolk injection
2.6 ng/kg D-CATH-2

Salmonella enteritidis
10-100
CFU/embryo

22h after infection

Schneider et al. (2016c)

D-CATH-2 via embryonic route of administration

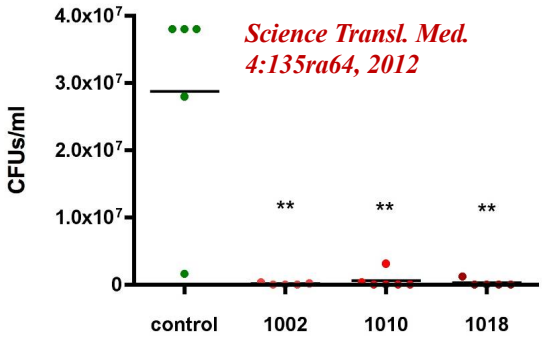
Target species	**Challenge**	**Result**	
Chicken 3 days pre-hatch	*E. coli* (i.t.) 7 days post hatch	Mortality Morbidity Bacterial load	30% 52% 93% ↓
Chicken 3 days pre-hatch	*S. enteriditis* (s.c.) 3 days post hatch	Mortality Morbidity	50% 67% ↓
Zebrafish 28 hpf	*S. enteriditis* 18-20 hpi	Mortality delayed	24 h

- **Peptide doses in models are too low to be directly antimicrobial**
- **Efficacy despite 6 day to 10 day 'gap' between treatment and challenge!**

New immunomodulatory peptides show broad protection in Mouse Model Infections

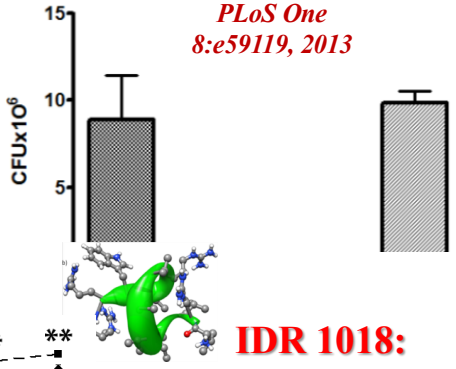
Observations by Hancock Laboratory (UBC, Vancouver)

Invasive *Staph.aureus* Mouse Model



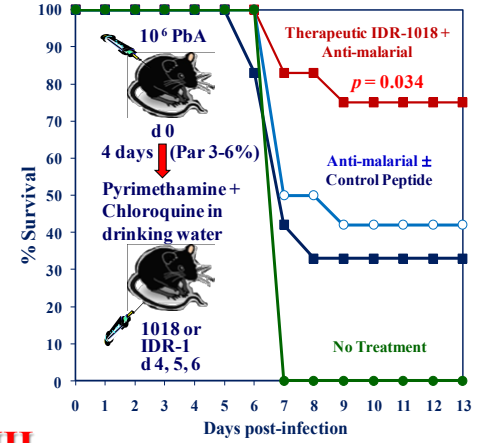
Science Transl. Med. 4:135ra64, 2012

MDR -TB Mouse Model

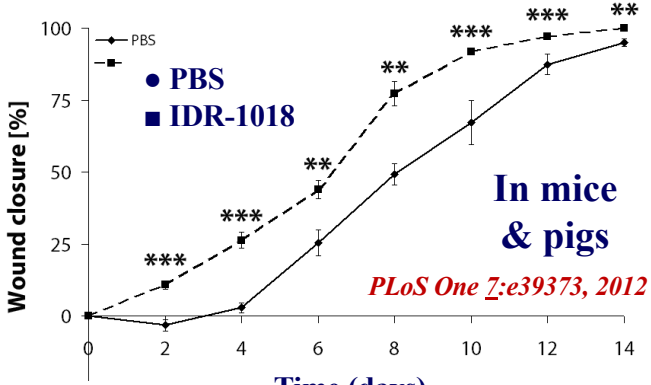


PLoS One 8:e59119, 2013

Cerebral Malaria Mouse Model



IDR 1018: $VRLIVAVRIWRR\text{-}NH_2$ *Science Transl. Med. 4:135ra64, 2012*



In mice & pigs

PLoS One 7:e39373, 2012

Grand Challenges in Global Health

Also protects vs. *E. coli, Salmonella, Klebsiella, Pseudomonas*, MRSA, Tuberculosis, Pox & HSV viruses

IBD, CF, Sterile inflammation; LPS/hypoxia-ischemia

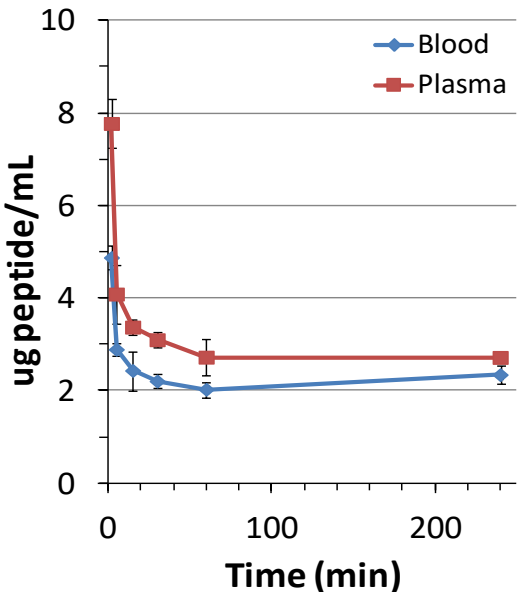
→ Wound Healing

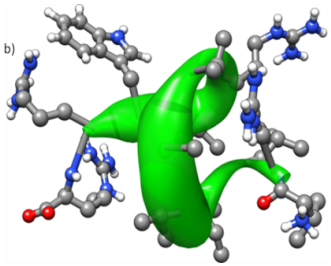
Lars Steinstraesser, Louis Schofield, Ariel Achtman, Bruno Rivas, Rogelio Hernandez Pando, Carina Mallard

Pharmacokinetics of IDR peptides

Hancock Laboratory (UBC, Vancouver)

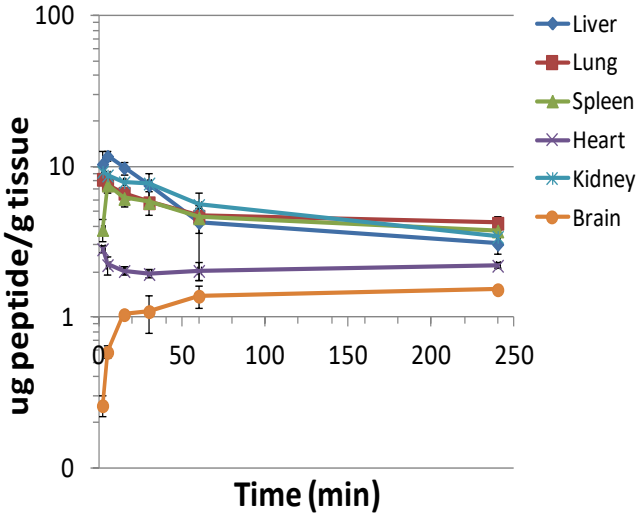
Pharmacology



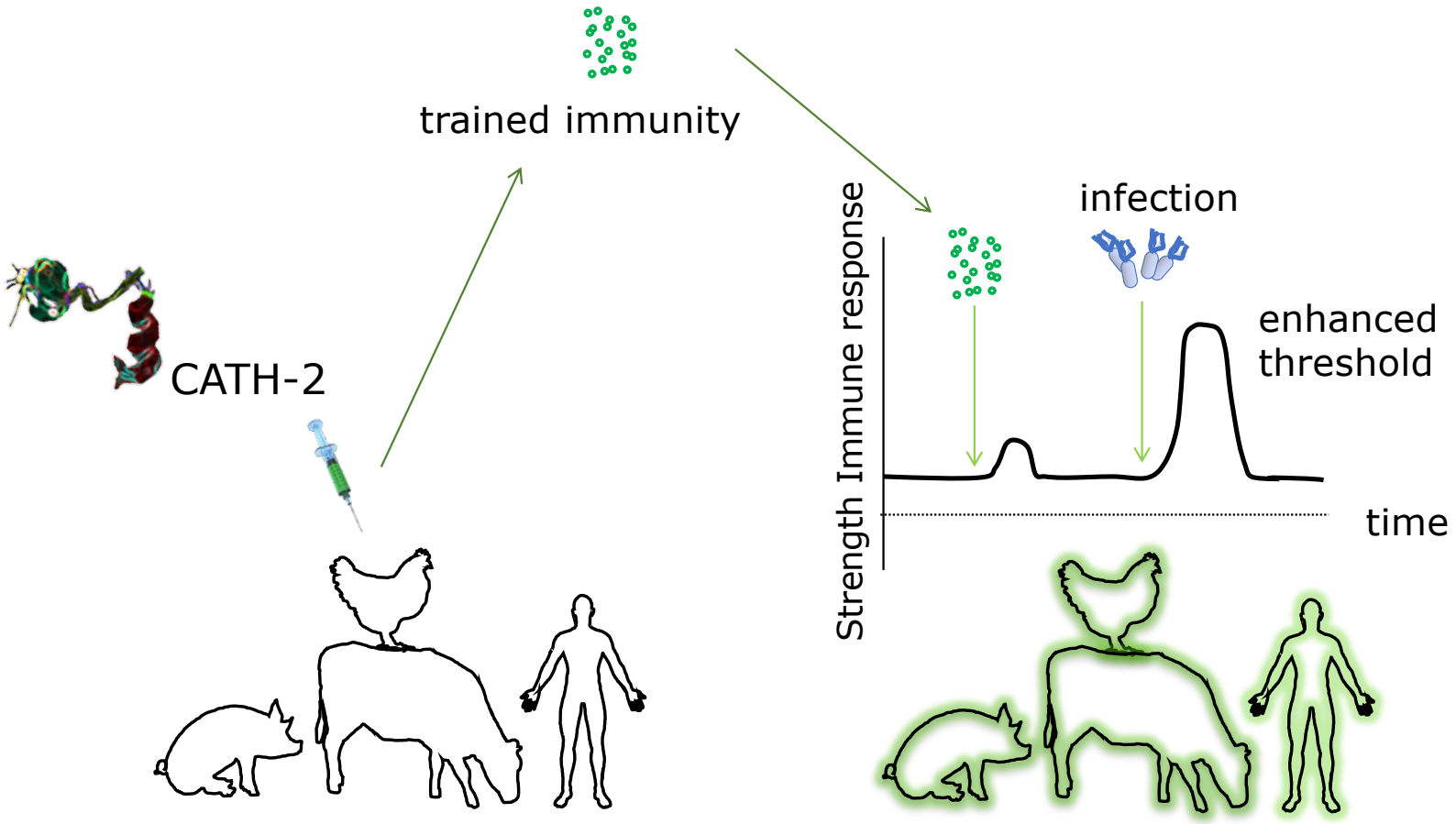


CENTRE FOR DRUG RESEARCH AND DEVELOPMENT

- Formulation (nanoparticles, etc)
- Improved design for certain properties (aggregation; stability)
- Animal Models (dosing)
- Animal Health – Alternatives to antibiotics



Peptide-induced innate immune memory?



Epidemiological observations of non-specific effects of vaccines

- Live vaccines induce cross-resistance
  - BCG
  - Measles-containing vaccines
  - Oral polio vaccines
  - Vaccinia against smallpox
- Inactivated vaccines induce cross-resistance
  - Diphteria-tetanus-pertussis containing vaccines
  - Hepatitis B vaccine
  - Inactivated polio vaccine

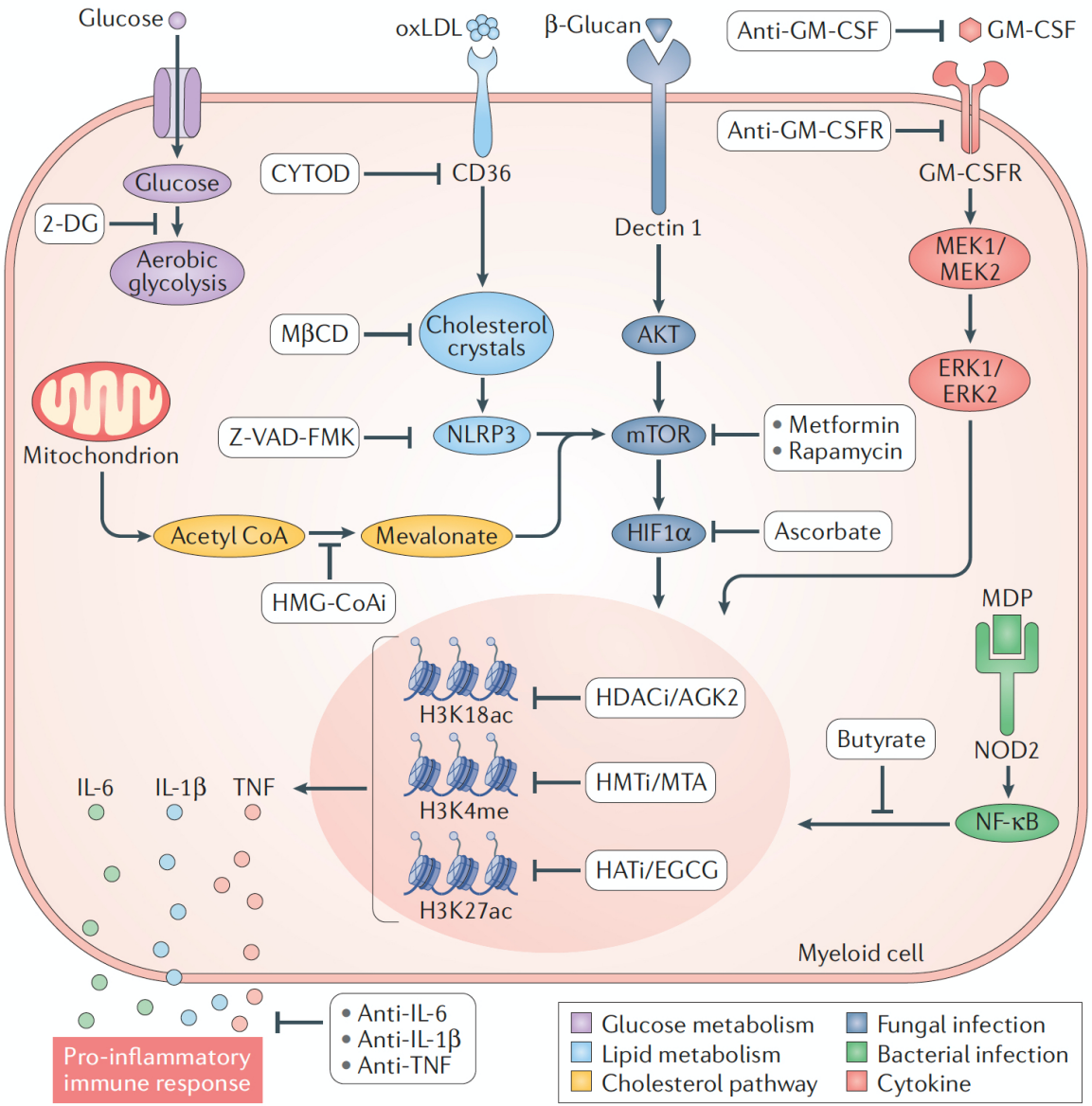
Jensen et al. (2016)

Pathogen-associated molecules and cross-protection

Component	Source	Cross-protection	Reference
LPS (endotoxin)	G(-) bacteria	*Staphylococcus aureus*	Breyne (2017)
Peptidoglycan (muramyl dipeptide)	Bacteria	Toxoplasma	Krahenbuhl (1981)
Flagellin	G(-) bacteria	*Streptococcus pneumoniae* Rotavirus	Munoz (2010)
FimH (adhesin)	*Escherichia coli*	Influenza virus	Abdul-Careem (2011)
β-glucan	Fungi	*S. aureus* *S. pneumoniae*	Marakalala (2013)
Chitin	Fungi	*S. aureus* *E. coli*	Rizzetto (2016)
CpG oligonucleotide	Bacteria (synthetic)	*E. coli* Influenza virus	Ribes (2014) Jiang (2011); Norton (2010)

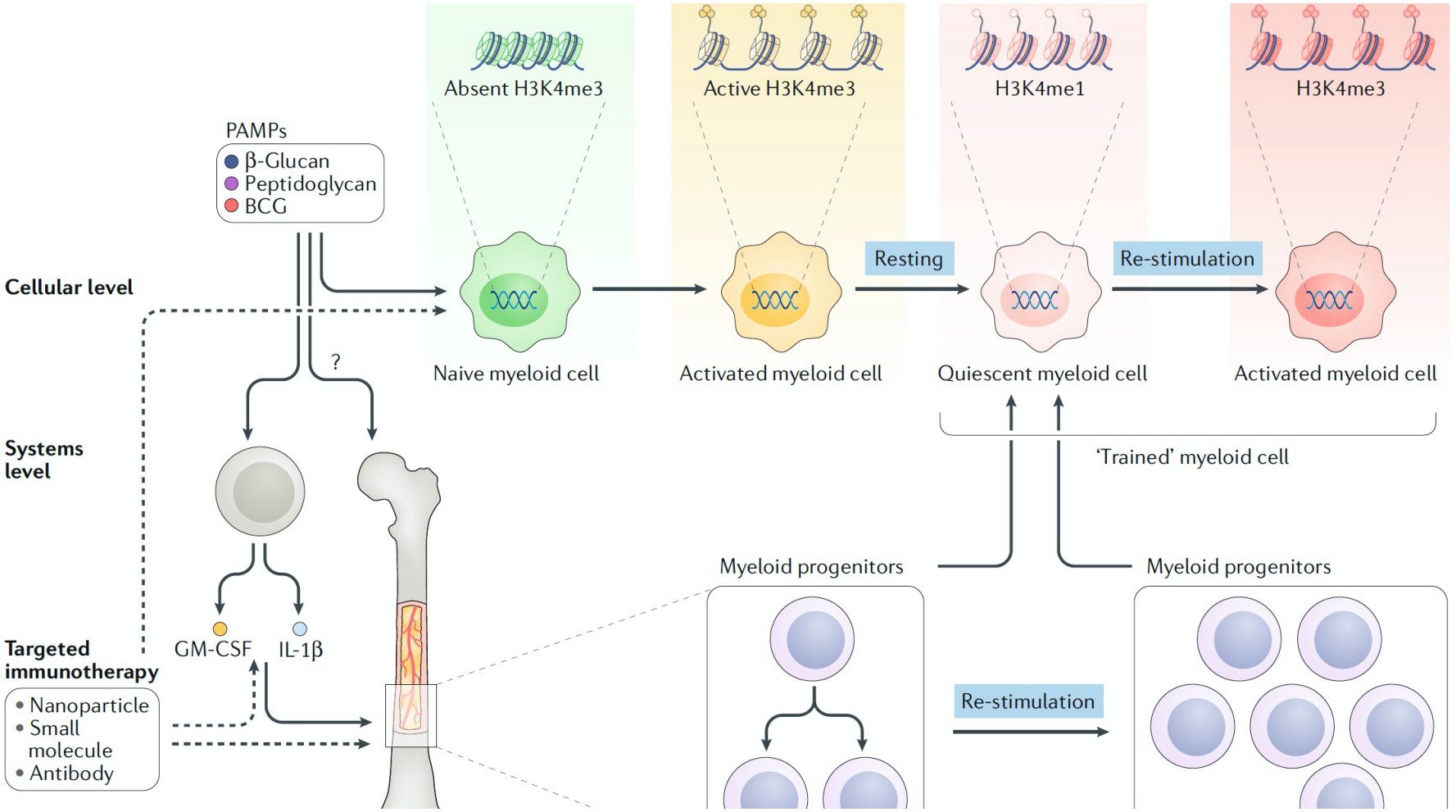
Adapted from Sánchez-Ramón et al.

Trained immunity regulatory pathways



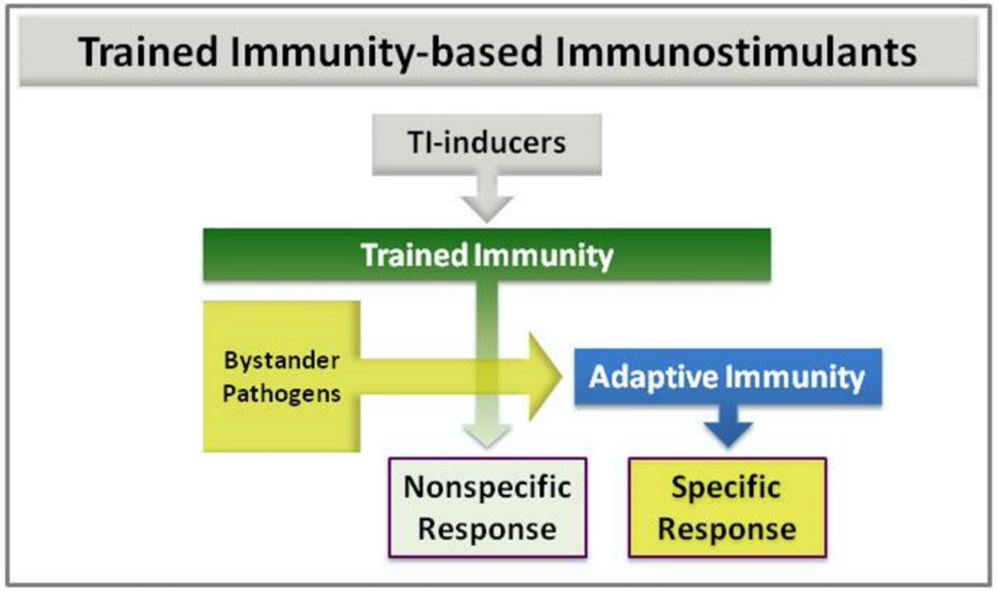
Mulder et al. (2019)

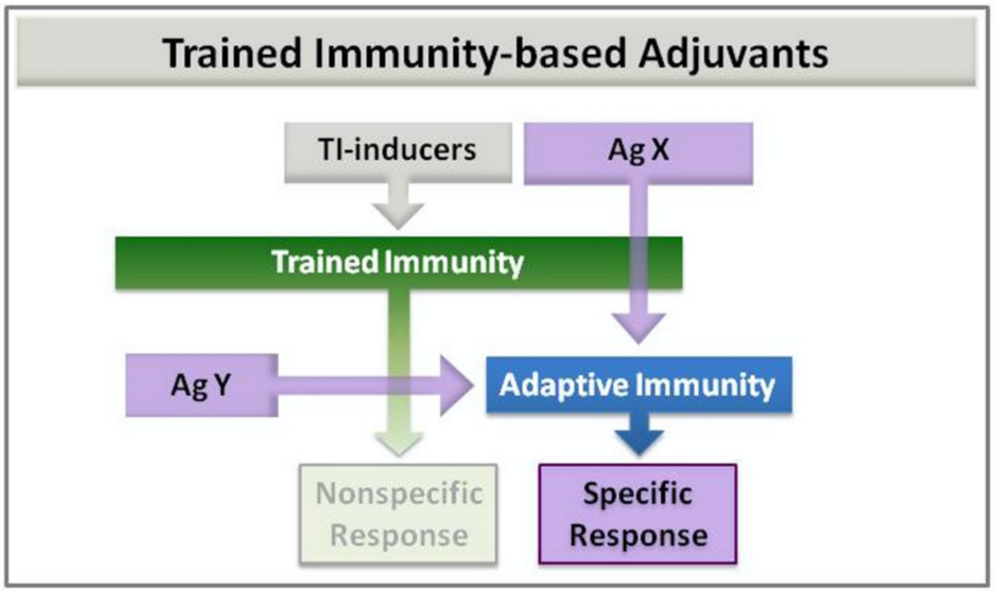
Trained immunity at the epigenetic, cellular and systems level



Mulder et al. (2019)

Application of trained immunity inducers





Sánchez-Ramón et al. (2018)

Applications of cathelicidin-derived peptides

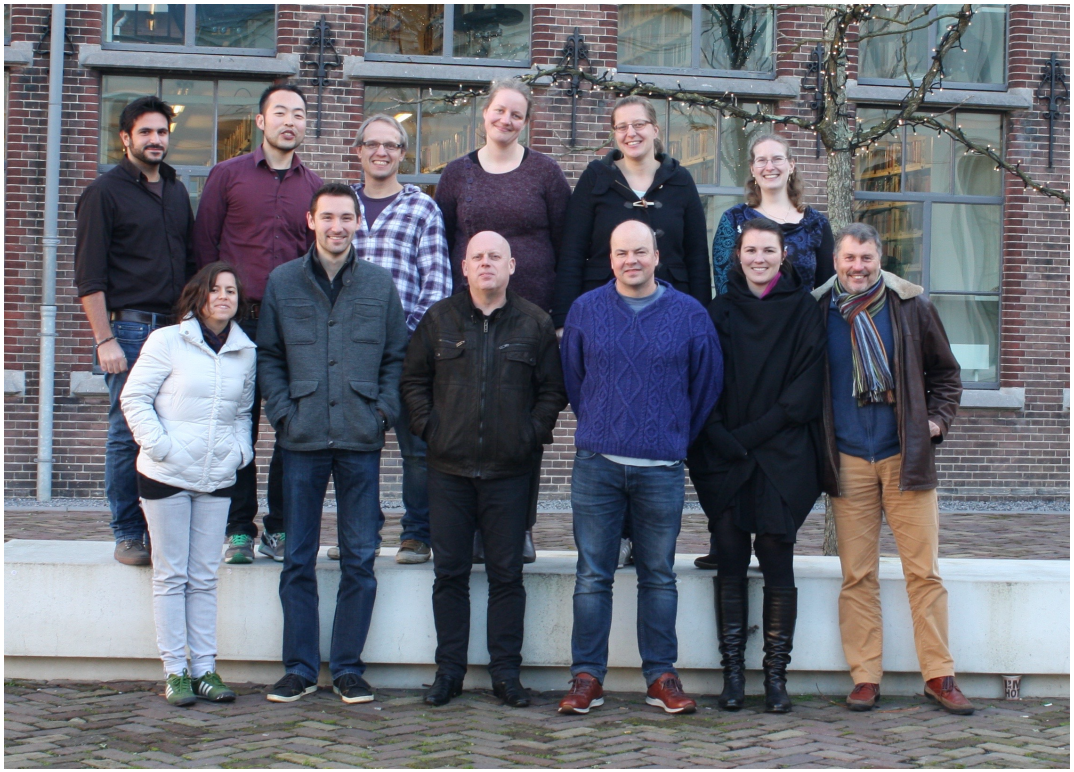
Therapeutic use:

- Direct antimicrobial activity
- As adjunct to antibiotics

Prophylactic use:

- Immunomodulation (generic protection)
  - mammals, fish, birds *(in ovo)*
- In vaccines as adjuvant

Division Molecular Host Defence



Andrea Bosso
Maarten Coorens
Tryntsje Cuperus
Soledad Ordonez
Viktoria Schneider
Weidong Zhang

Albert van Dijk
Martin van Eijk
Marina Kraaij
Maaike Scheenstra

Hanne Tjeerdsma
Edwin Veldhuizen