Universiteit Utrecht

Diergeneeskunde

Cathelicidins:

Host defense peptides against *E. Coli* infection

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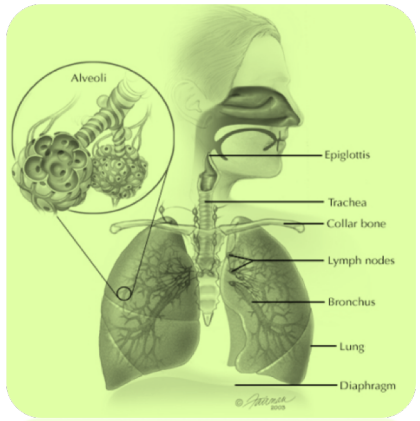
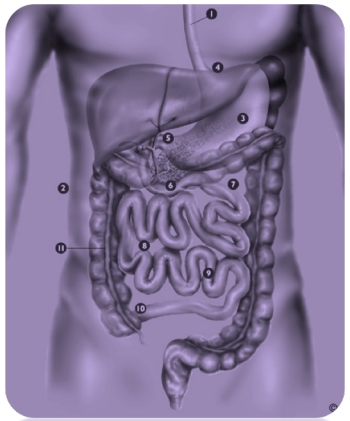
Organization of presentation

- Introduction: Host Defense Peptides (HDPs)
- Cathelicidins: structure and properties
- Cathelicidins: immunomodulatory antimicrobials
- Antimicrobial strategies involving HDPs
  - HDP-derived antimicrobials
  - HDP-derived immunomodulators
- Conclusions

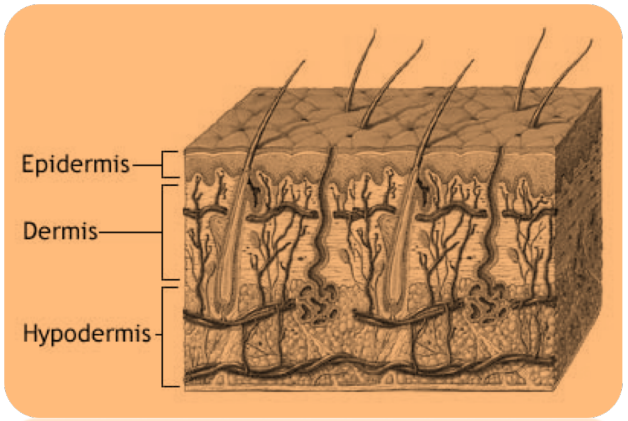
Interfaces with the outside world



150 m^2



400 m^2

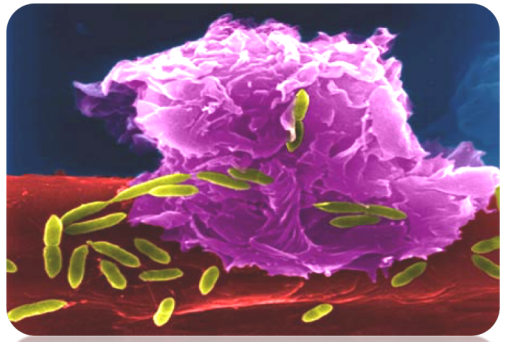


2 m^2

Innate Host Defense

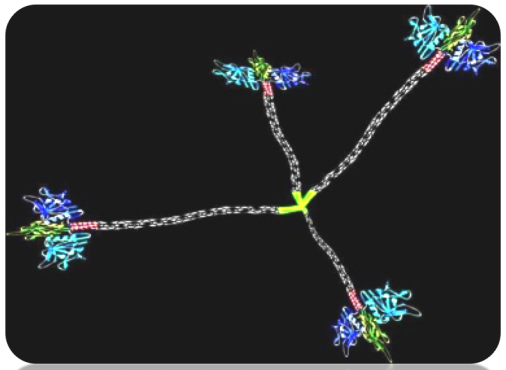
- Present in all organisms
- Limited repertoire of molecules
- Rapid
- Broad specificity
- Ancient

The first line of defense against infections



Cellular defenses

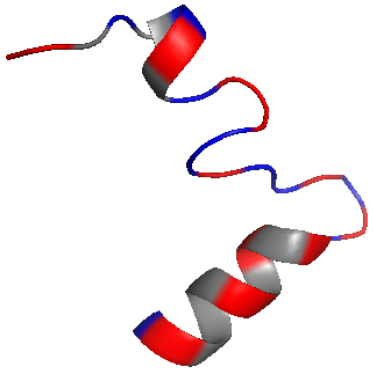
Neutrophils (heterophils)
Macrophages, NK cells



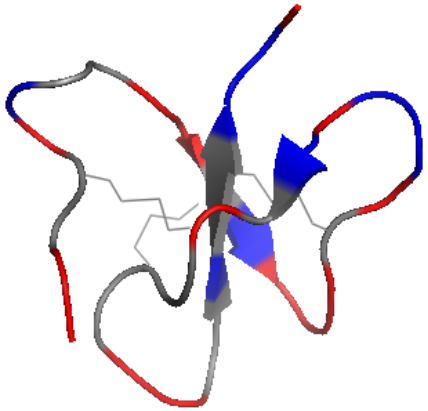
Effector molecules

Enzymes, **Host defense peptides**, Collectins

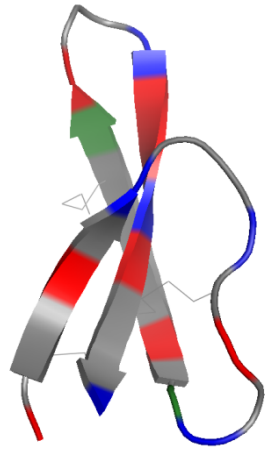
Examples of vertebrate host defense peptides



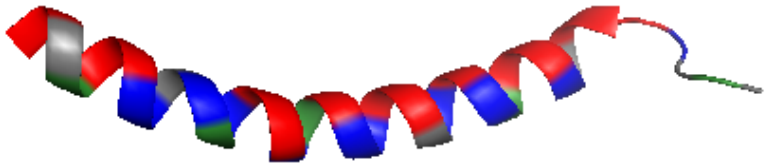
CATH-2



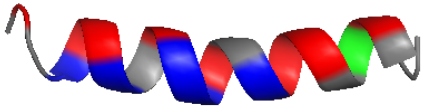
HBD-2



HD-5



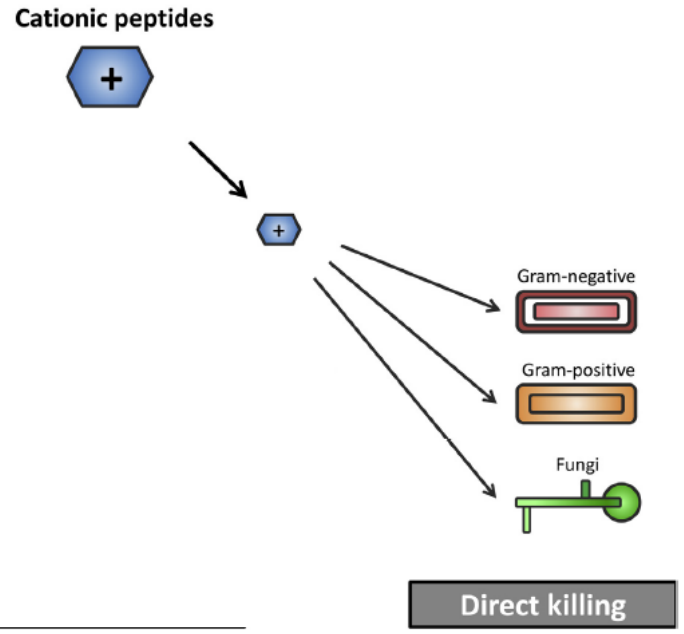
LL-37



Magainin-2

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

Functions host defense peptides



Cuperus *et al*. 2013

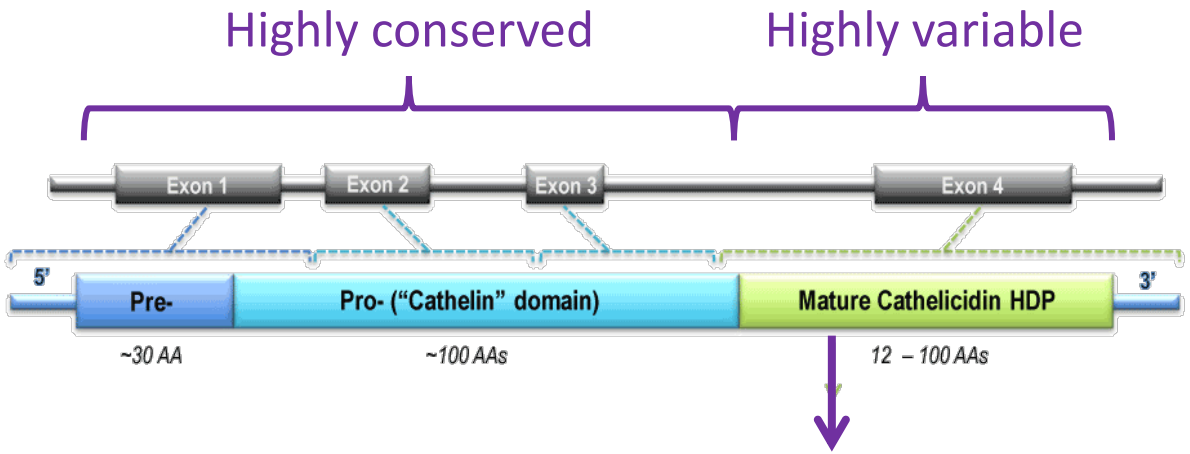
Cathelicidins: structures and properties

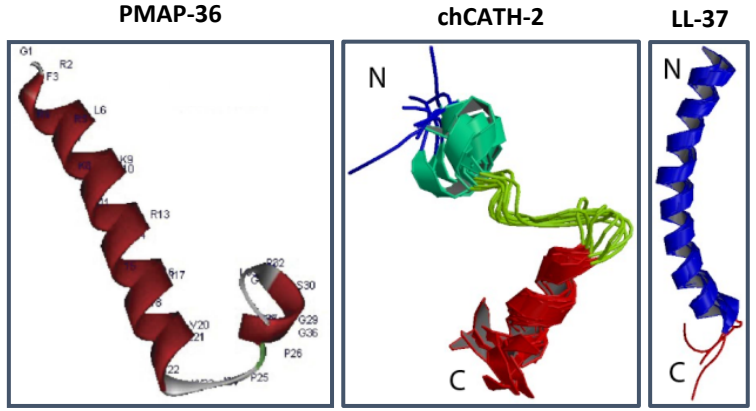
Characteristics

- Present in most studied vertebrates
- Produced by neutrophils, epithelial cells, monocytes, NK cells

- Formed as pre-pro-peptide
  - Small
  - Cationic
  - Amphipathic

- Antibacterial
- Immunomodulatory





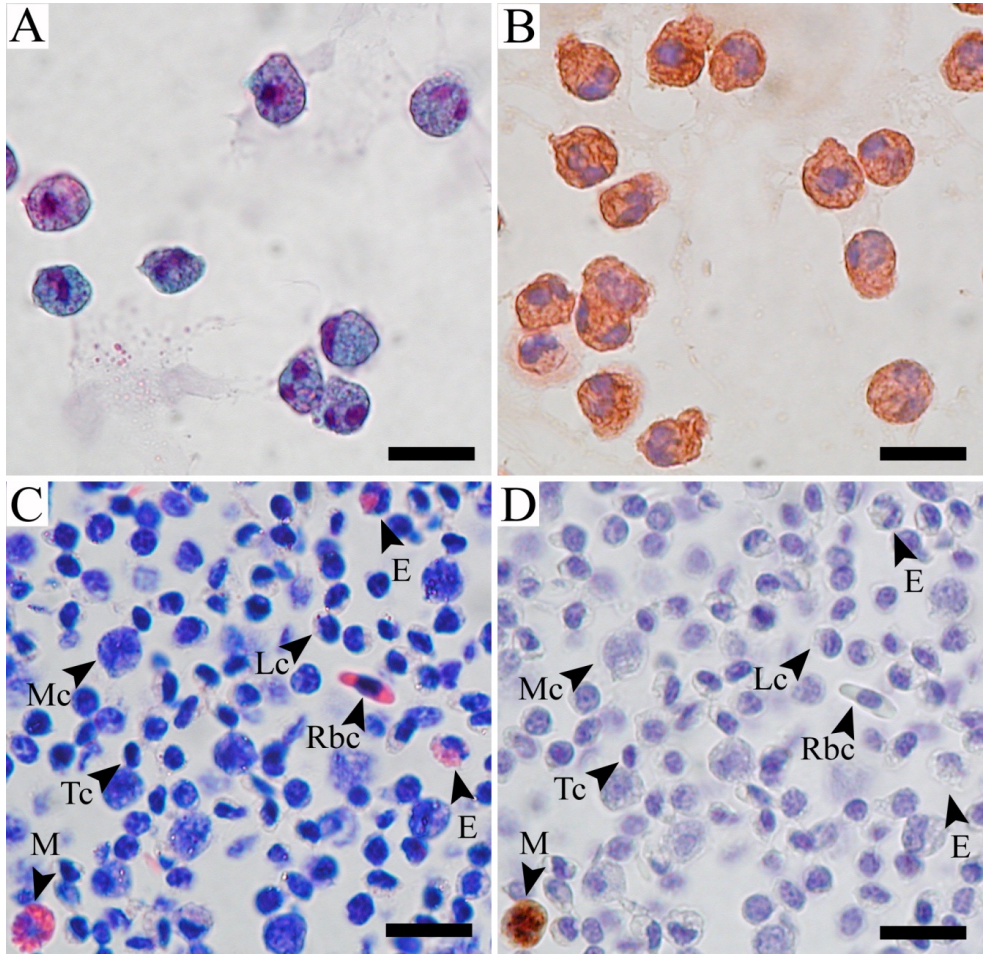
Storici, P, *et al.* FEBS lett, 1994; van Dijk, *et al.* Mol Immunol, 2009; Mishra, B. *et al.* RSC Adv, 2013

Linde *et al.* 2013l
Van Dijk, *et al.* 2009

CATH-2 is produced by chicken heterophils

Heterophils

Mononuclear cells

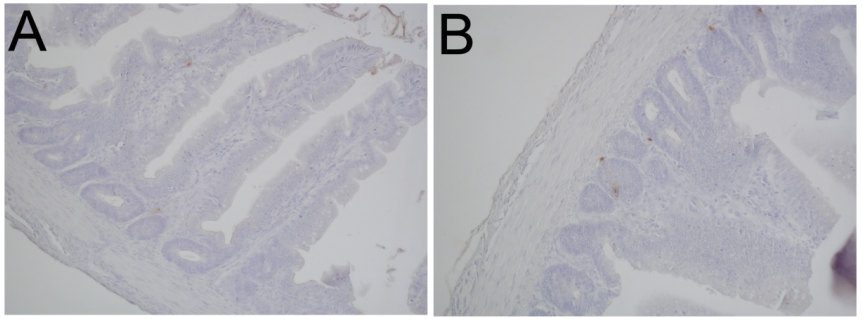


Giemsa **Anti-CATH-2**

Van Dijk et al., 2009a

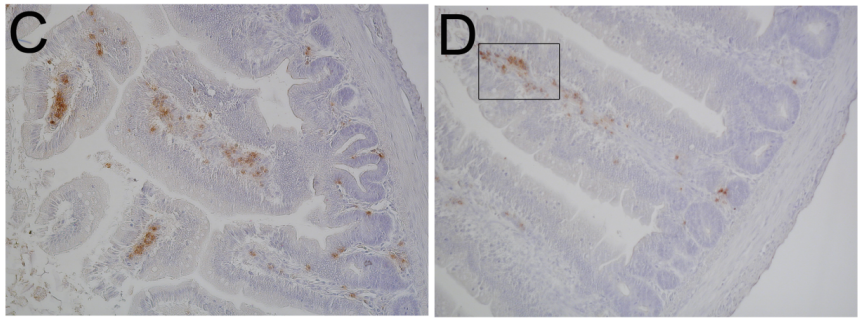
Salmonella enteritidis challenge of chickens results in recruitment of CATH-2 containing heterophils

Control
8h p.i.



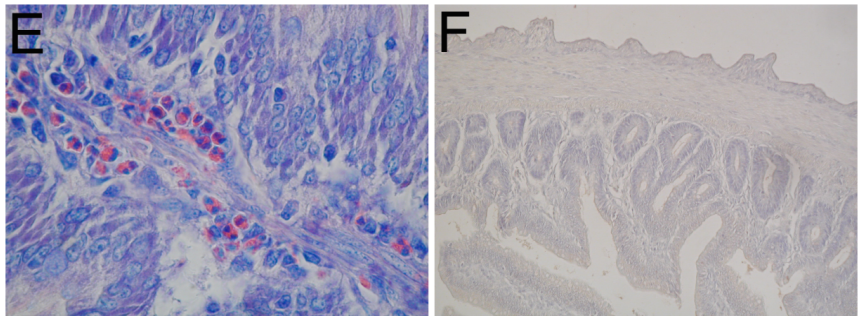
Control
48h p.i.

S. enteritidis
8h p.i.



S. enteritidis
48h p.i.

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

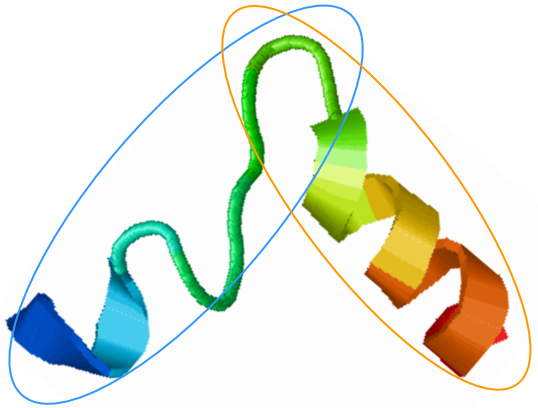


S. enteritidis
48h p.i.
(preimmune)

Van Dijk et al., 2009a

Antimicrobial activities of CATH-2

Truncated & substituted CATH-2 analogs



C1-27 (CATH-2) RFGRFLRKIRRFRPKVTITIQGSARFG

α — Hinge region — α

Truncated analogs

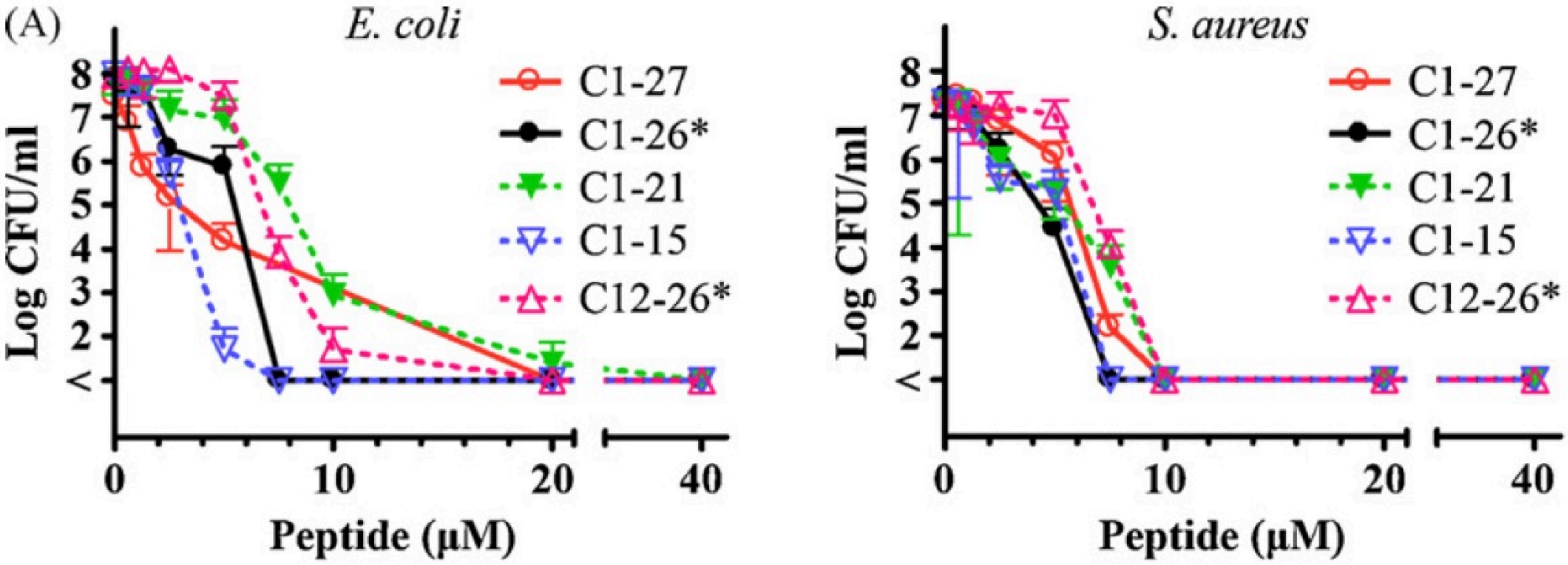
C1-26*	RFGRFLRKIRRFRPKVTITIQGSARF-NH2
C1-26	RFGRFLRKIRRFRPKVTITIQGSARF
C1-21	RFGRFLRKIRRFRPKVTITIQ
C1-15	RFGRFLRKIRRFRPK
C12-26*	FRPKVTITIQGSARF-NH2

Hinge region

C1-26(P>G)	RFGRFLRKIRRFRGKVTITIQGSARF
C1-26(P>L)	RFGRFLRKIRRFRLKVTITIQGSARF

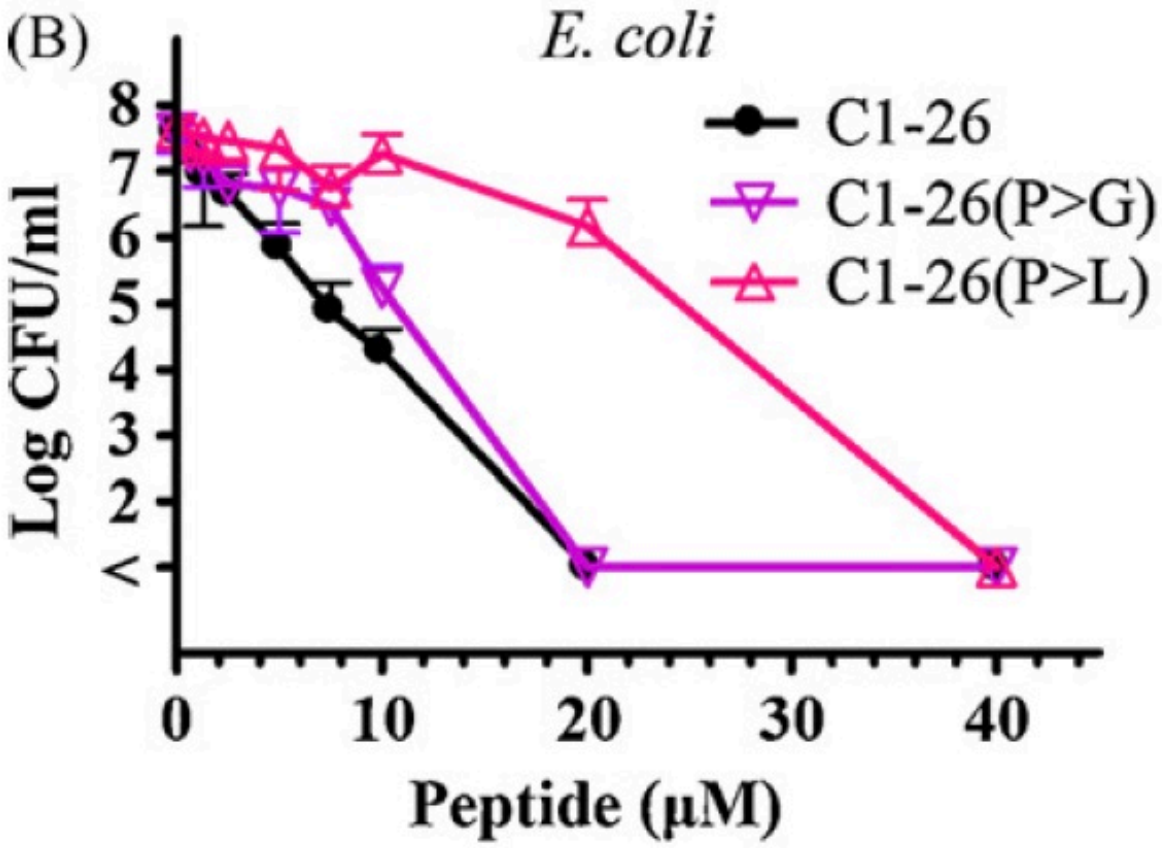
Albert van Dijk

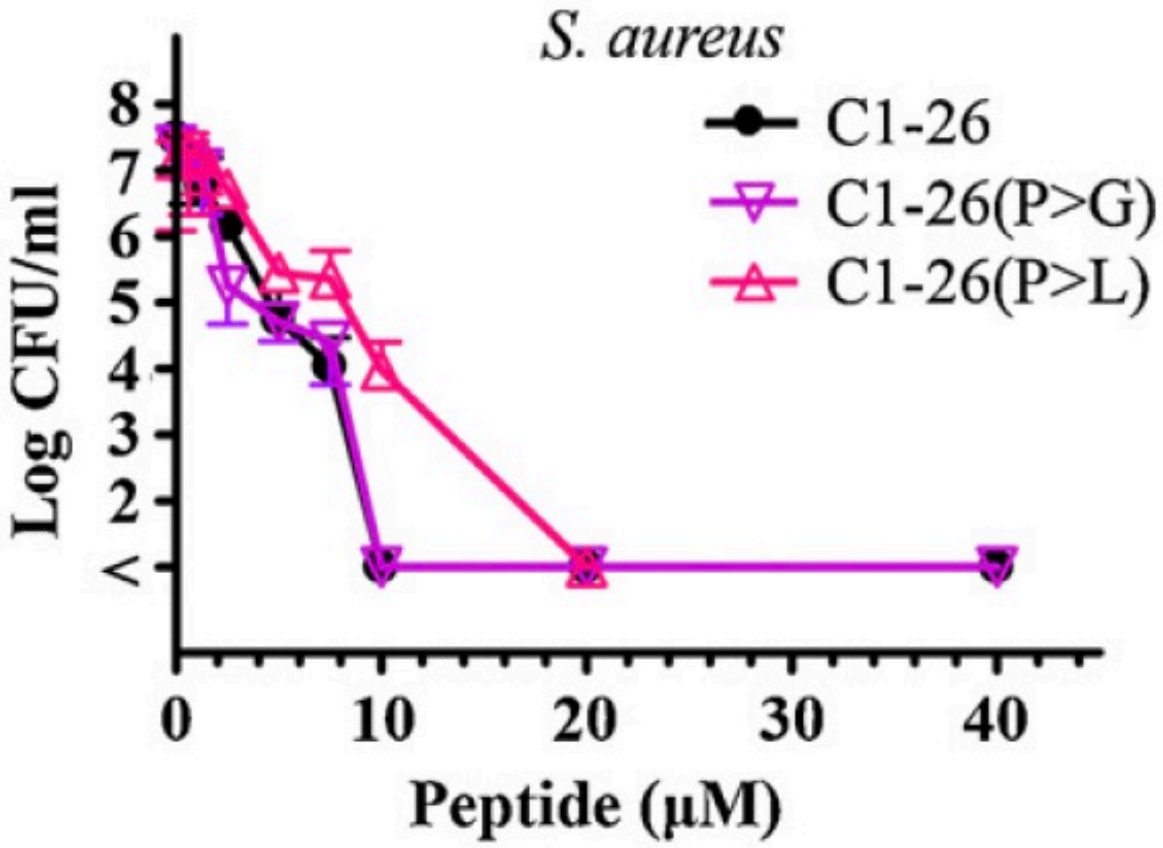
Antibacterial activities of truncated CATH-2 analogs



Van Dijk et al., 2009b

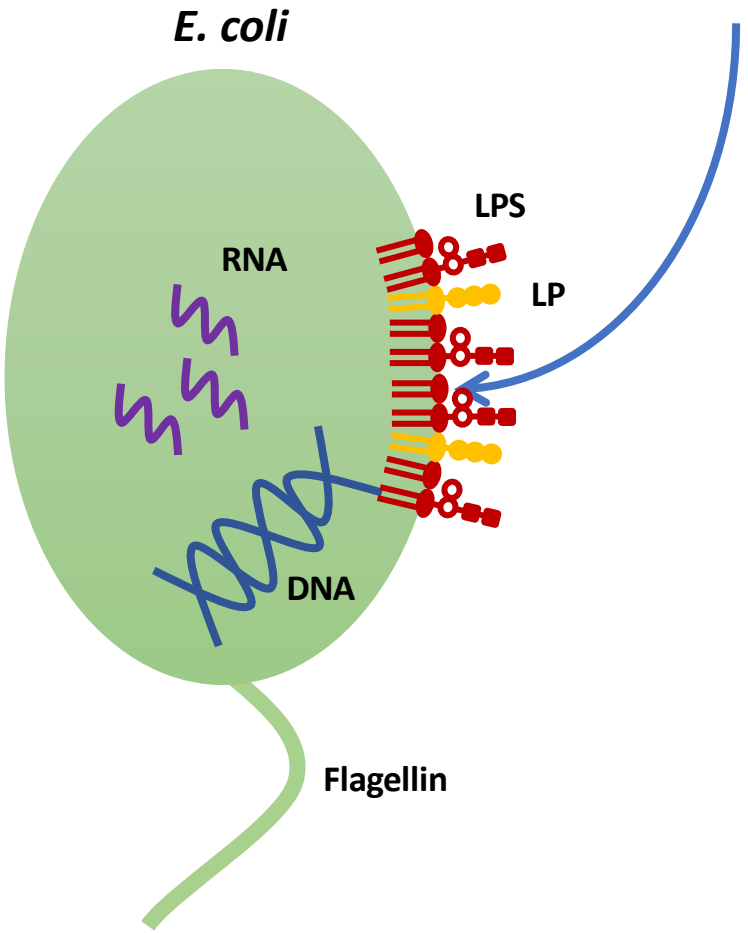
Antibacterial activities of CATH-2: role of hinge

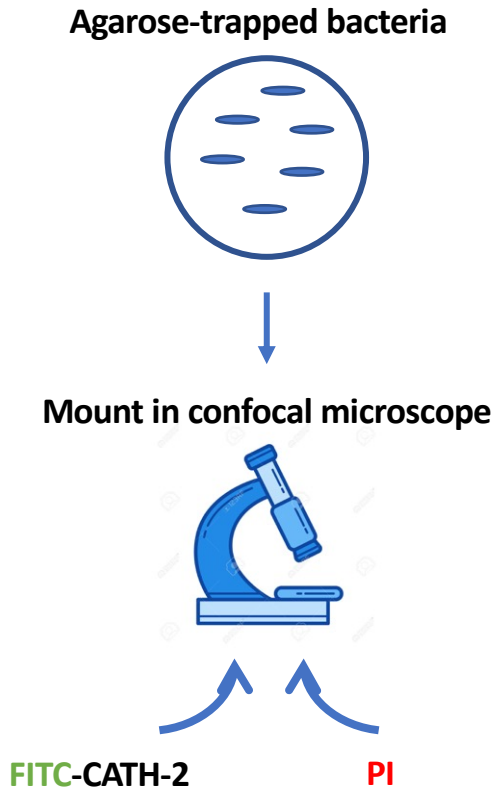




Van Dijk et al., 2009b

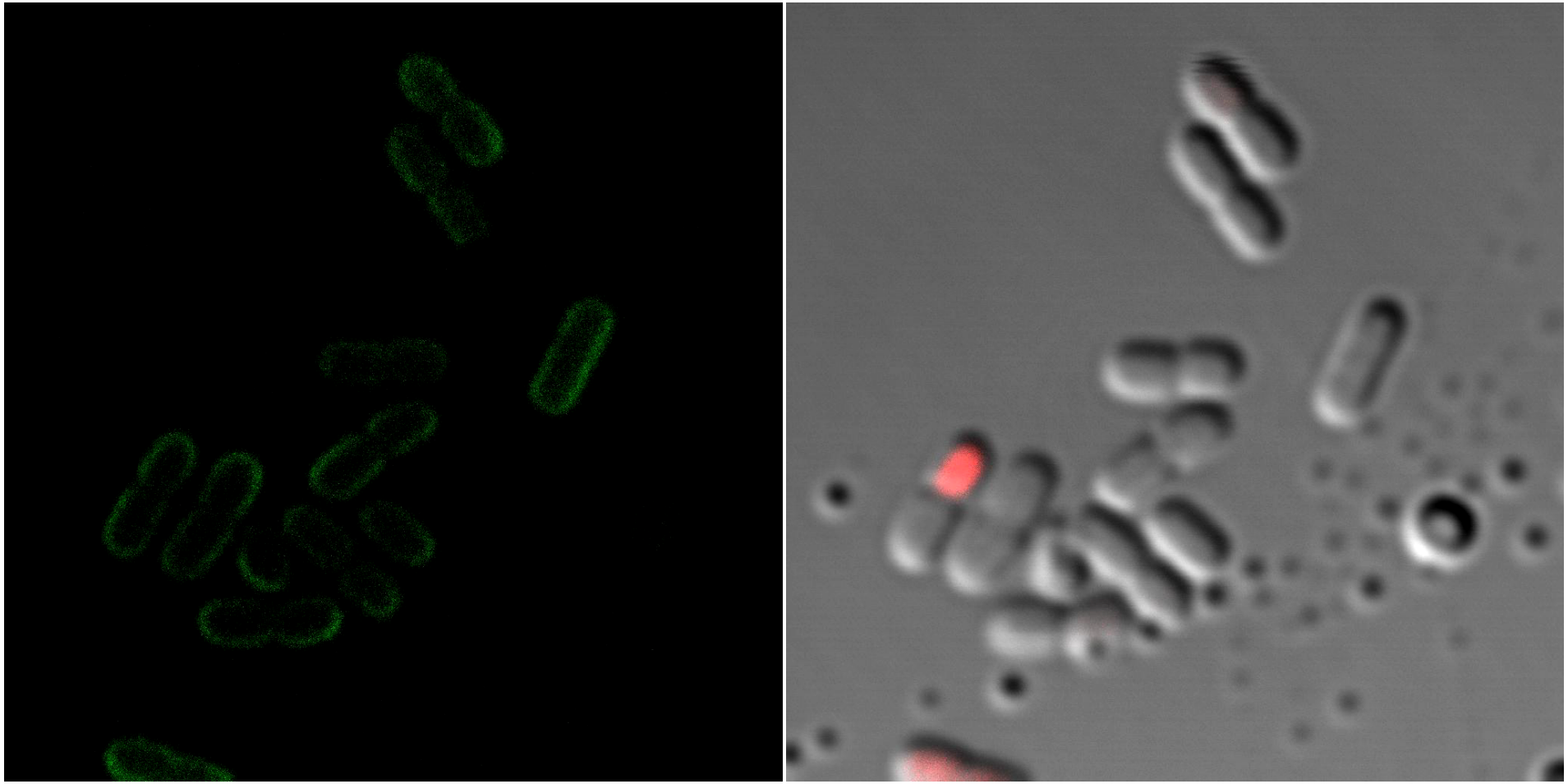
Visualization CATH-2 / *E. coli* interactions





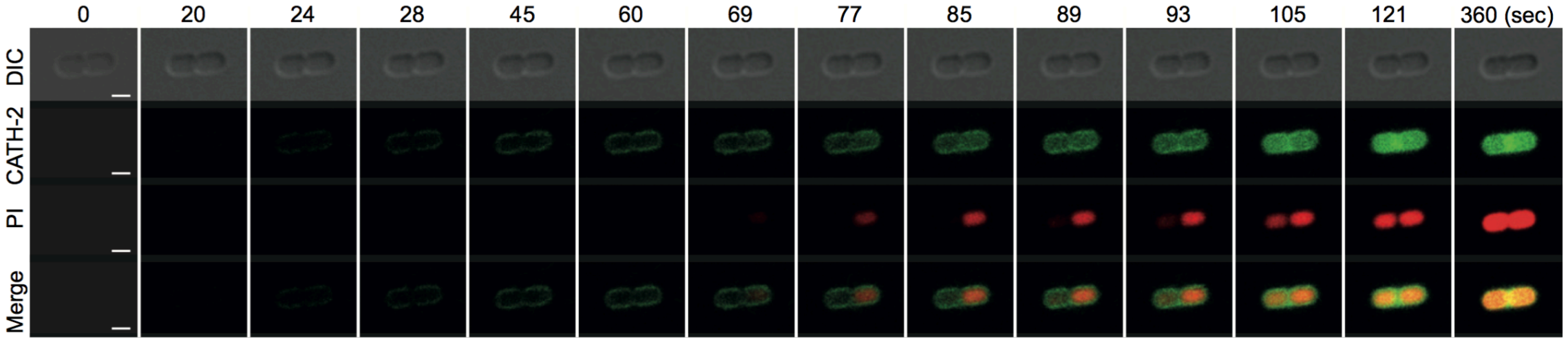
Viktoria Schneider

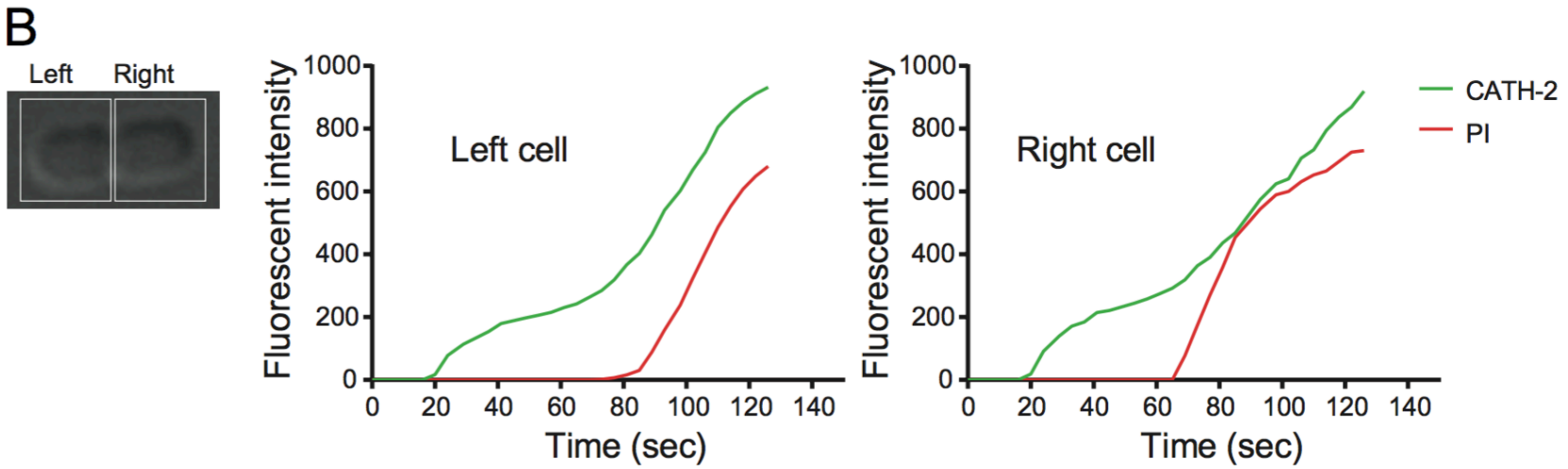
Live imaging of CATH-2-mediated *E. coli* killing

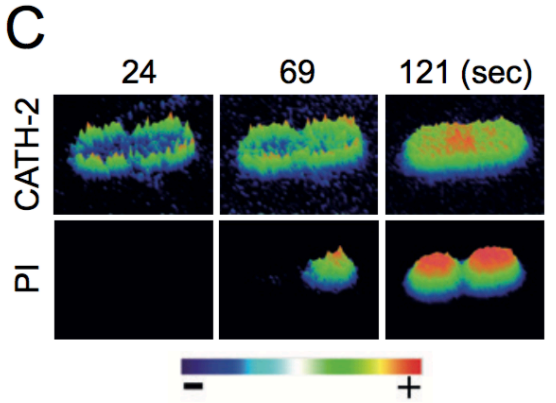


Schneider et al., 2016

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

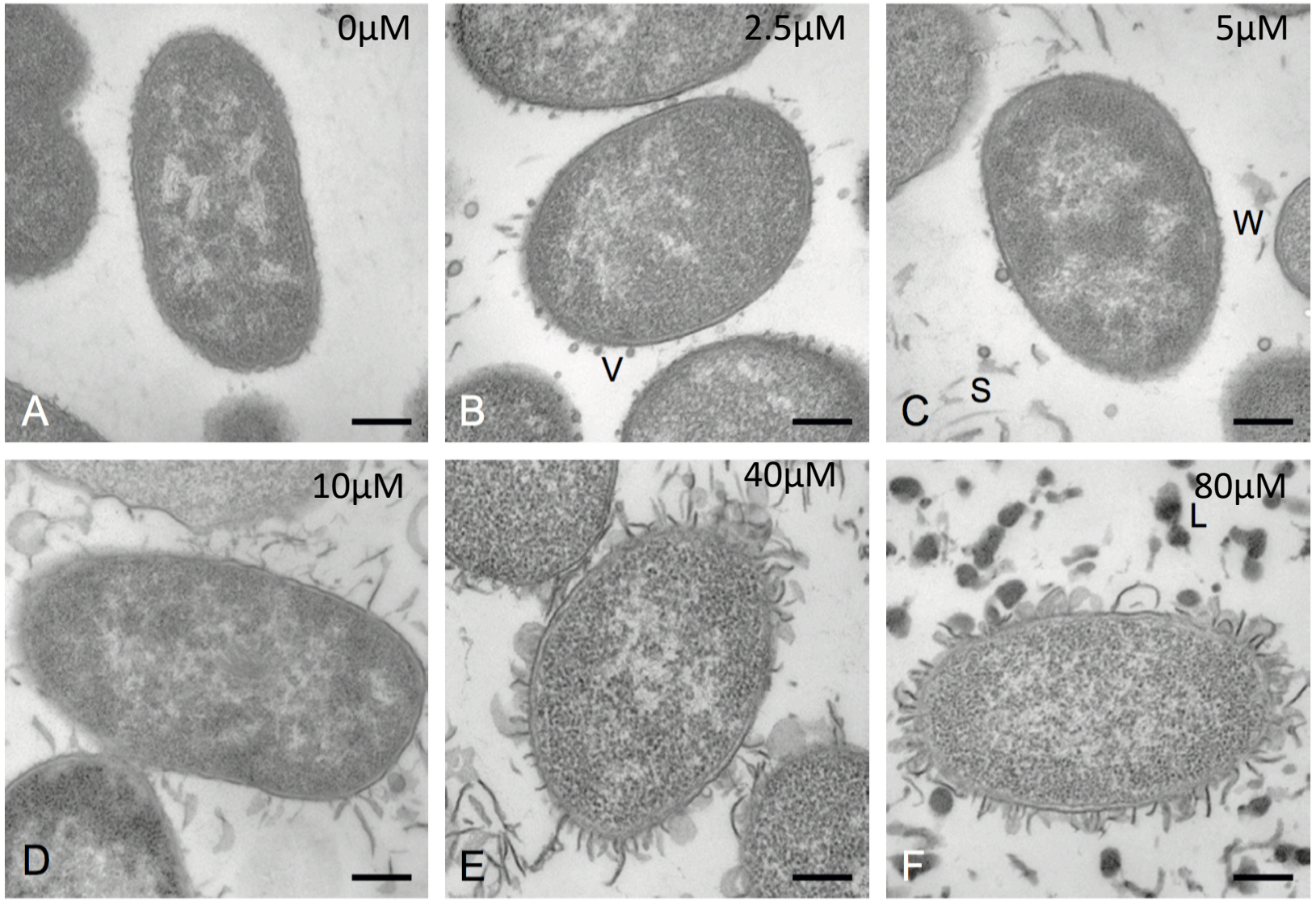






Schneider et al., 2016

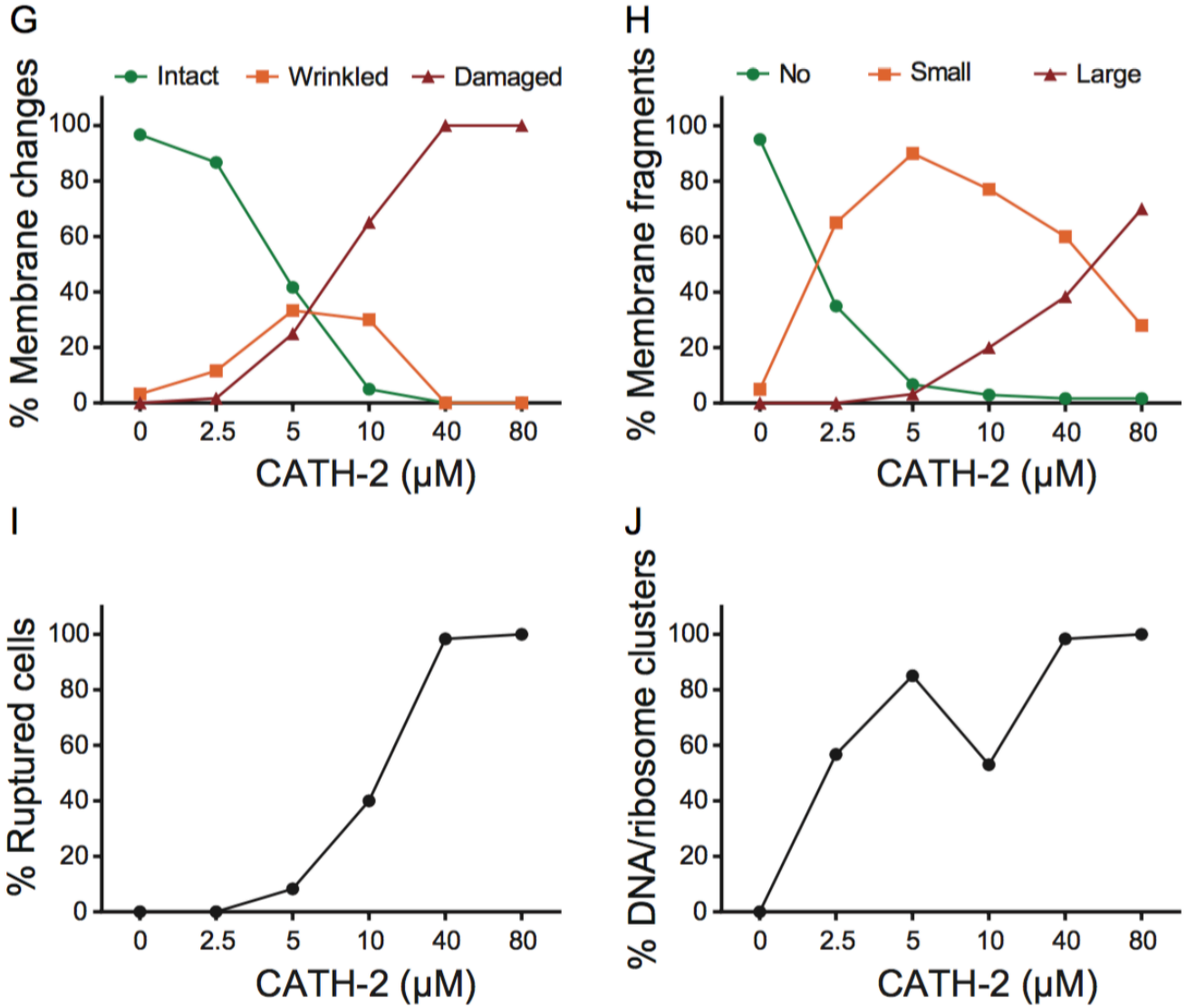
CATH-2 induced morphological changes *of E. coli*



30 min

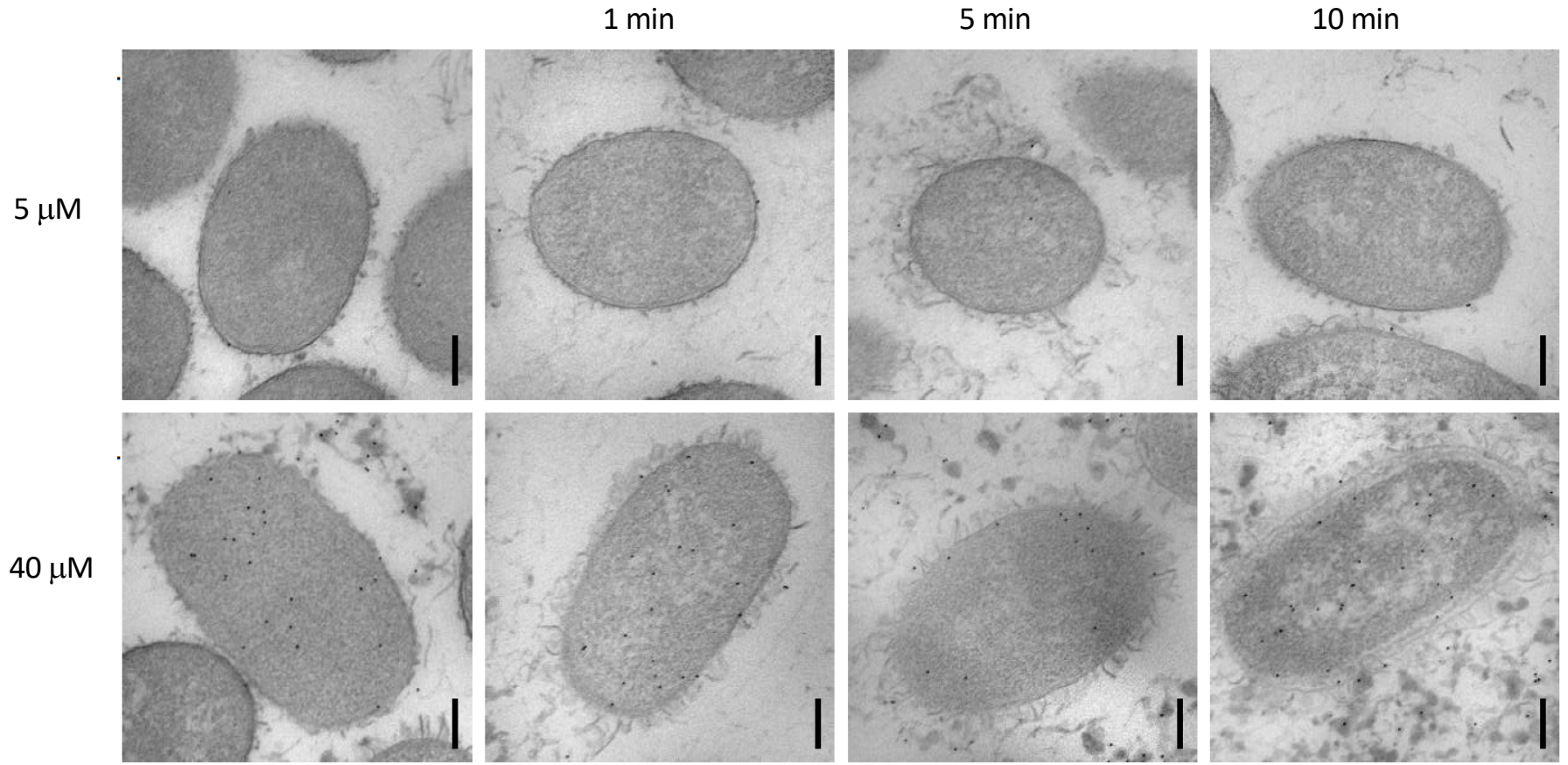
Schneider et al., 2016

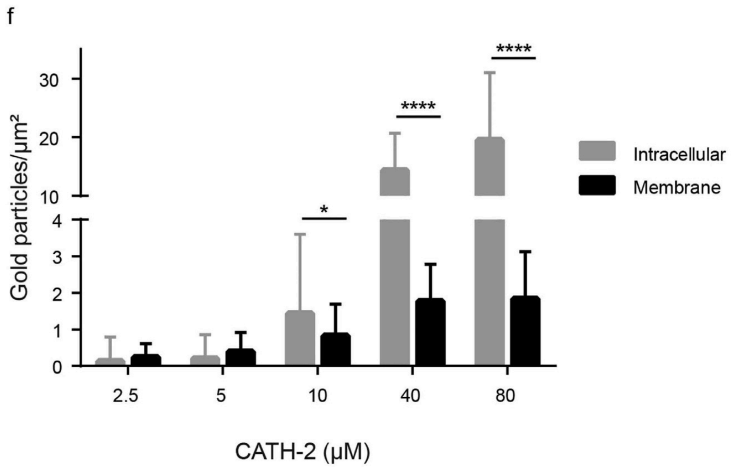
CATH-2 induced morphological changes of *E. coli*



Schneider et al., 2016

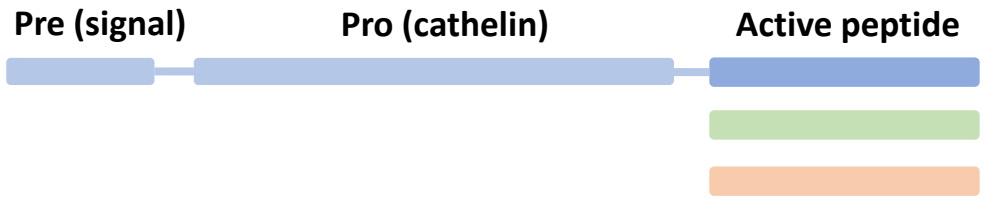
Localization of CATH-2 in *E. coli* determined by immuno-gold labelling





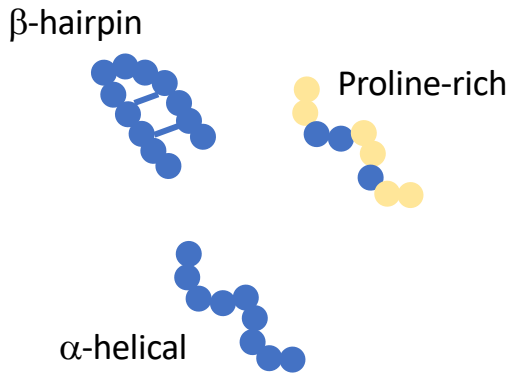
Schneider et al., 2016

Cathelicidins: modulating the inflammatory response

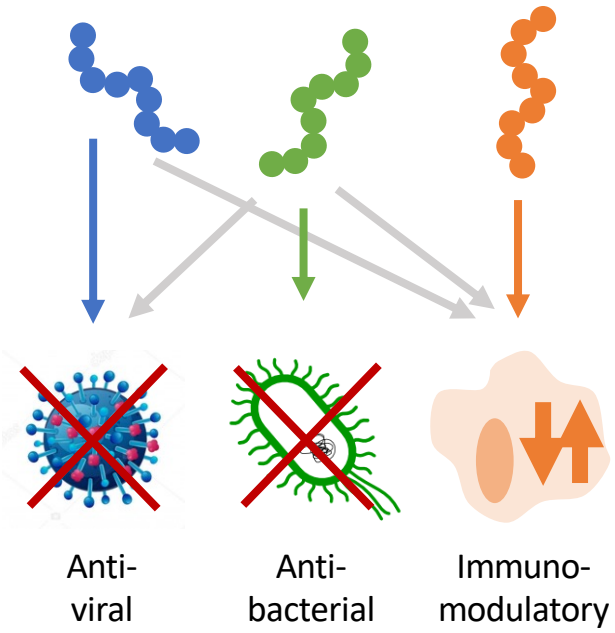


Difference in numbers

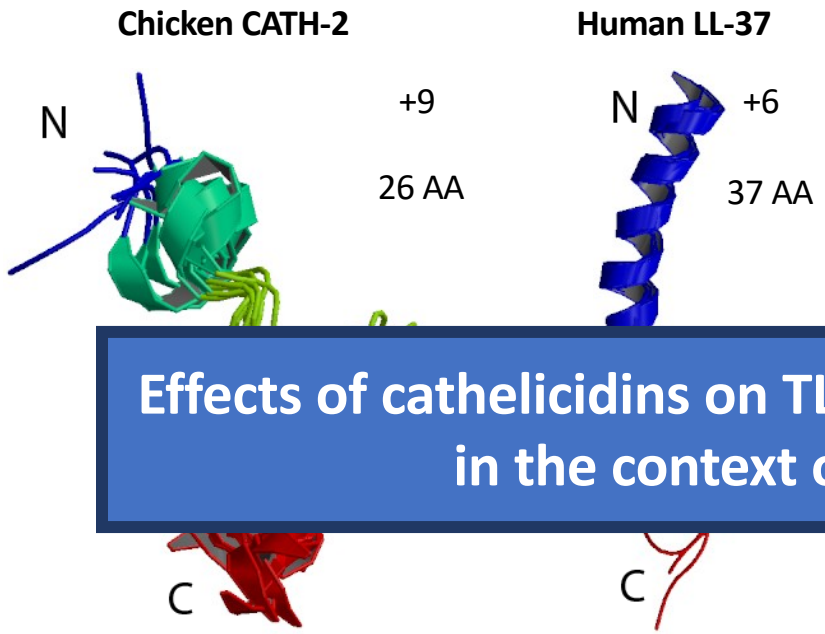
Difference in structures



Difference in functions

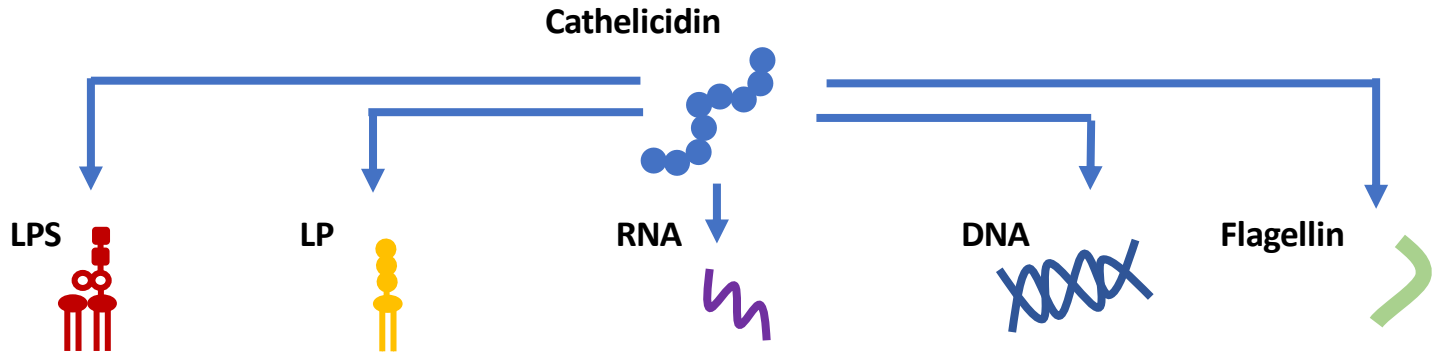


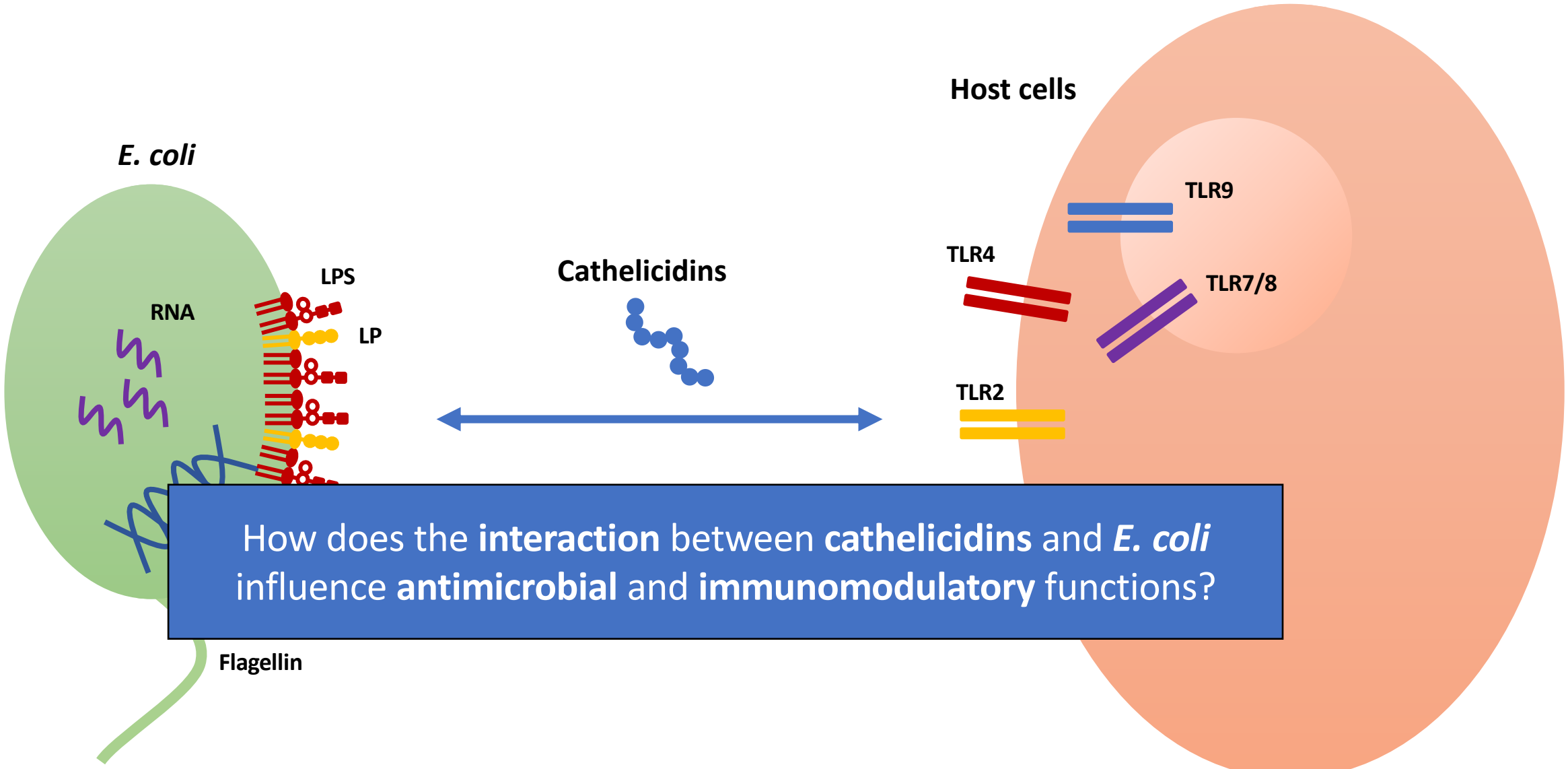
CATH-2 (chicken) vs. LL-37 (human)



Function	CATH-2	LL-37
Antimicrobial (MHB)	✓	✓
Antimicrobial (RPMI + FCS)	✓	✗
LPS neutralization (TLR4)	✓	✓
Lipoprotein neutralization (TLR2)	✓	✓
Improved Flagellin responses (TLR5)	?	✓

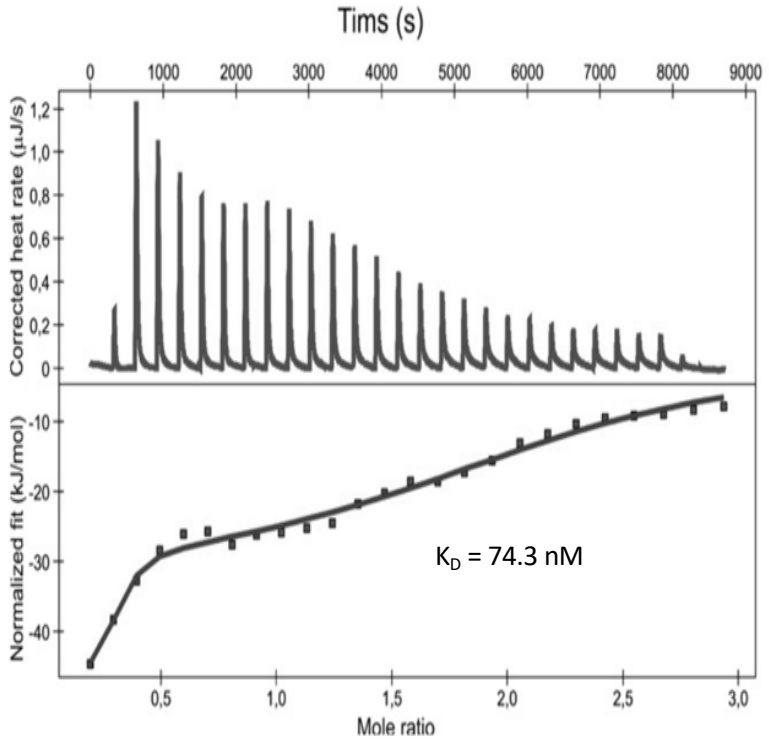
Effects of cathelicidins on TLR activation are mostly investigated in the context of purified TLR ligands…



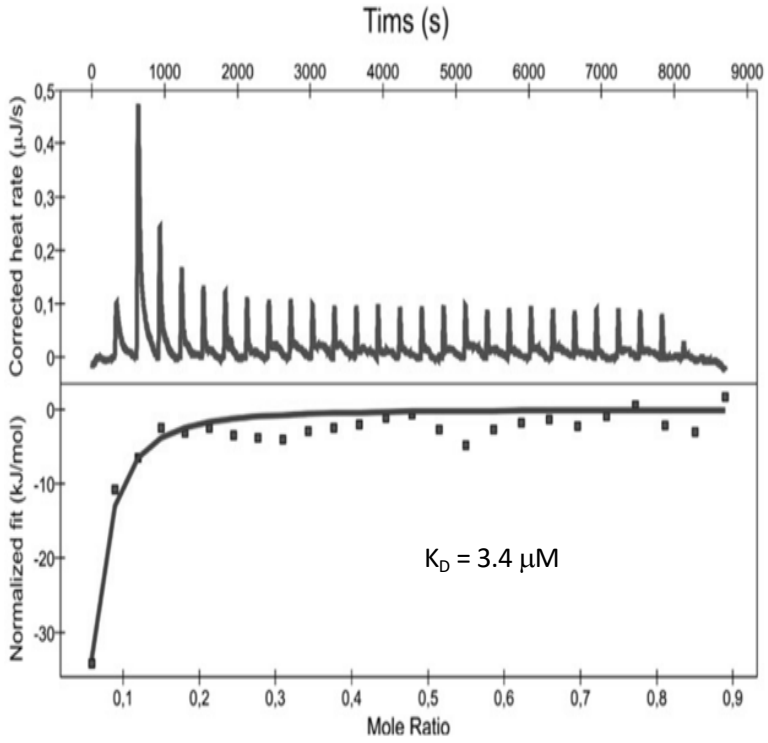

Host cells
E. coli
TLR9
TLR4
TLR7/8
LPS
Cathelicidins
RNA
LP
TLR2
How does the **interaction** between **cathelicidins** and ***E. coli*** influence **antimicrobial** and **immunomodulatory** functions?
Flagellin

CATH-2 binds to LPS, lipoproteins and DNA as determined by isothermal titration calorimetry

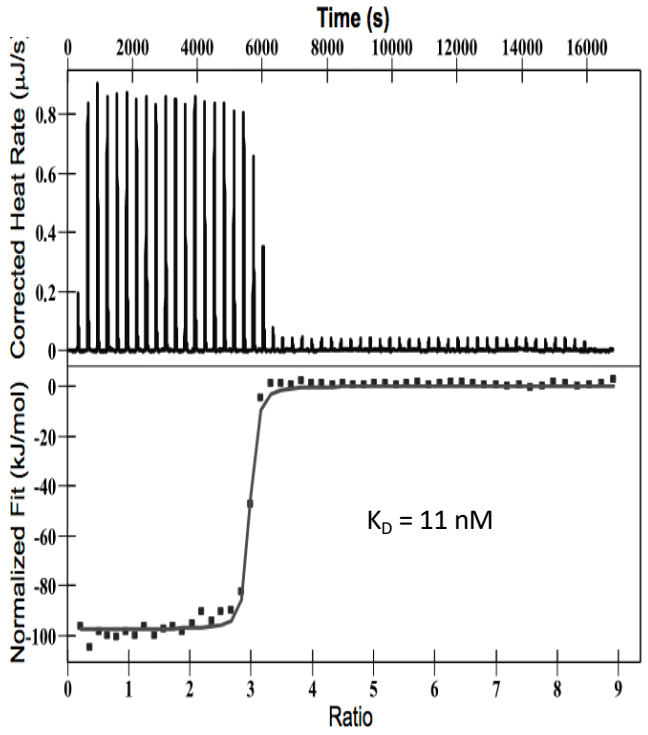
LPS O111:B4



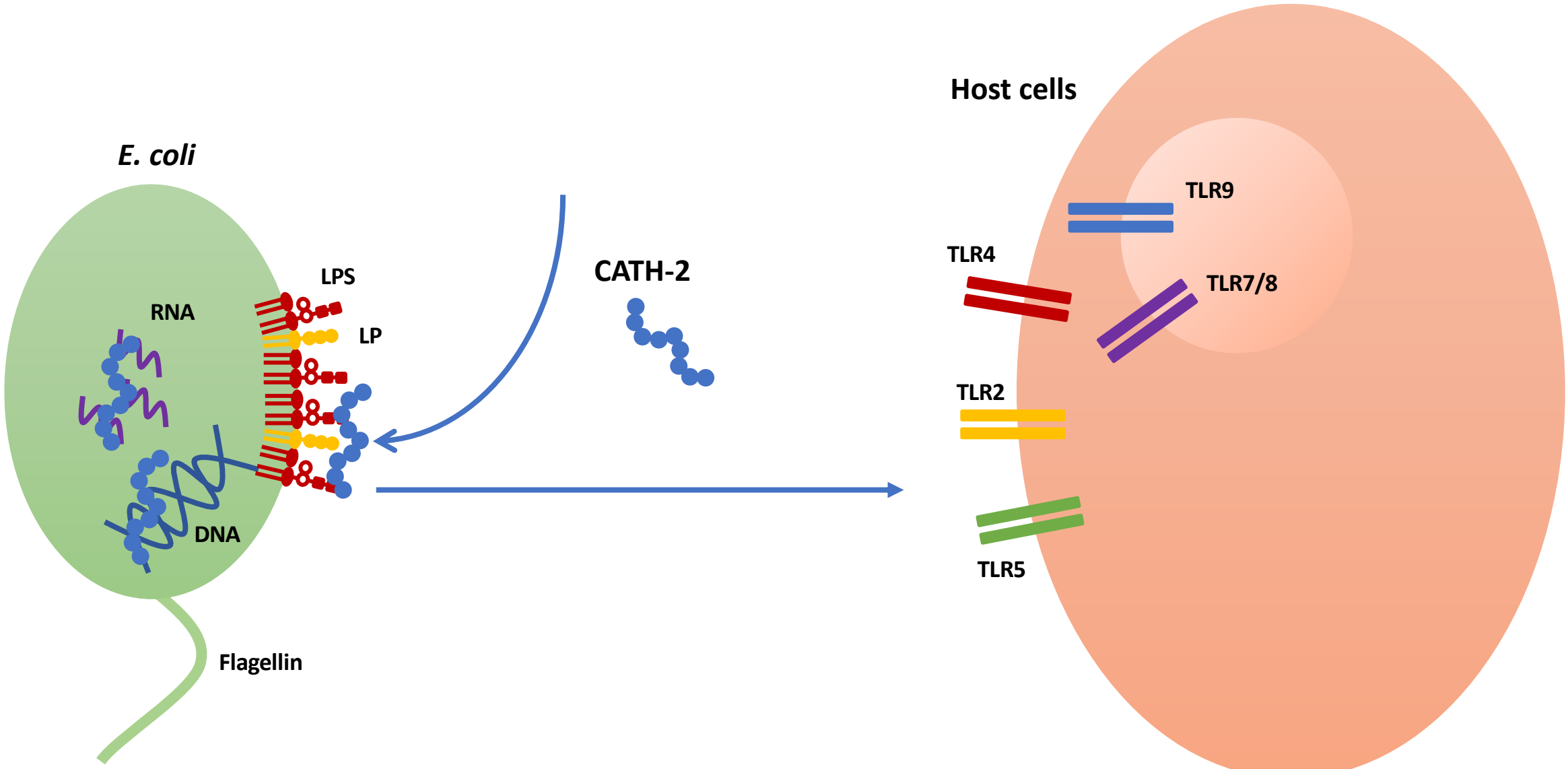
Pam_3CSK_4 (LP)



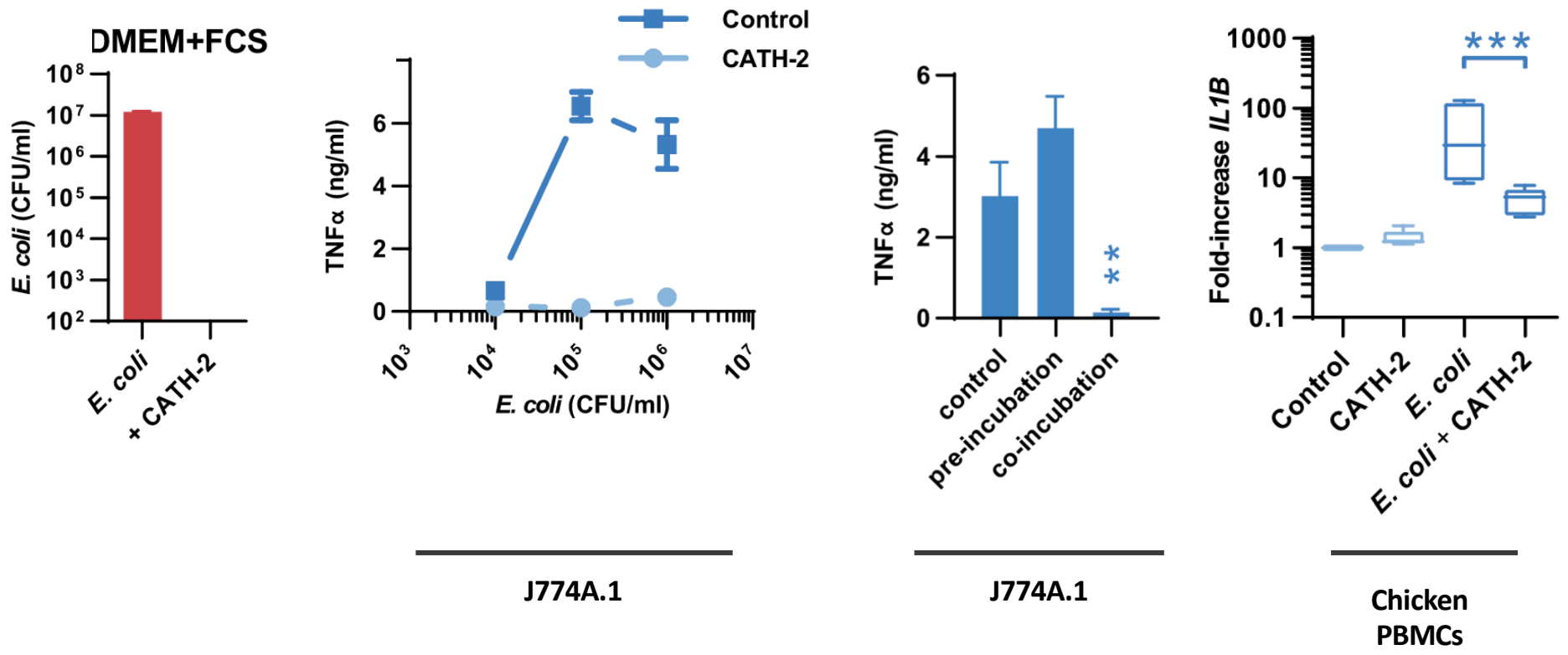
ODN-2006 (DNA)



Coorens et al., 2017

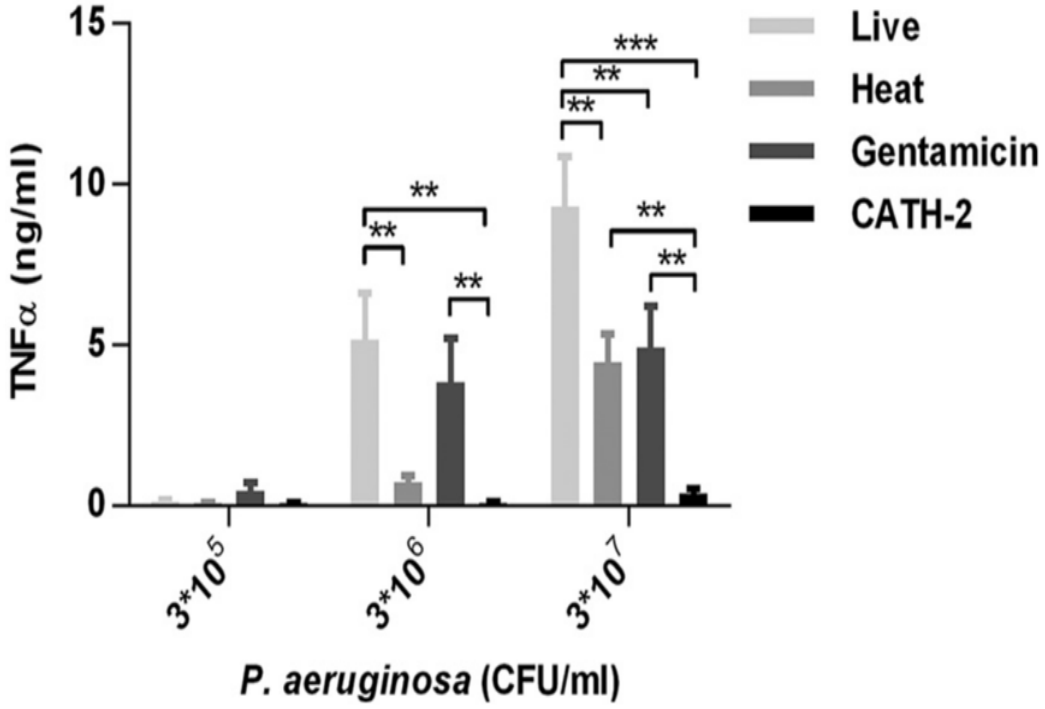

E. coli
LPS
LP
RNA
DNA
Flagellin
CATH-2
Host cells
TLR9
TLR4
TLR7/8
TLR2
TLR5

CATH-2 “silently” kills *E. coli in vitro*



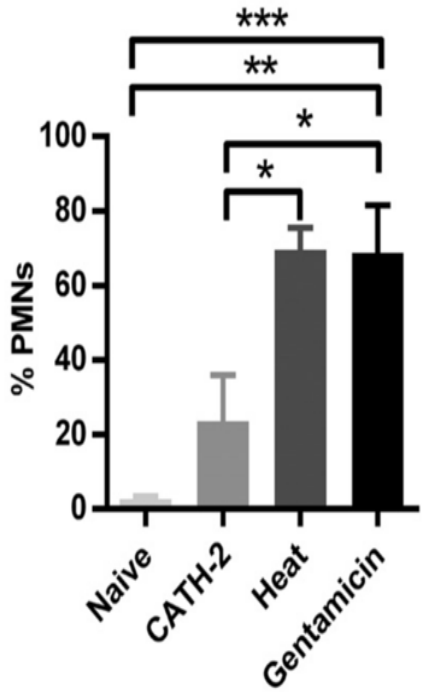
Coorens et al., 2017

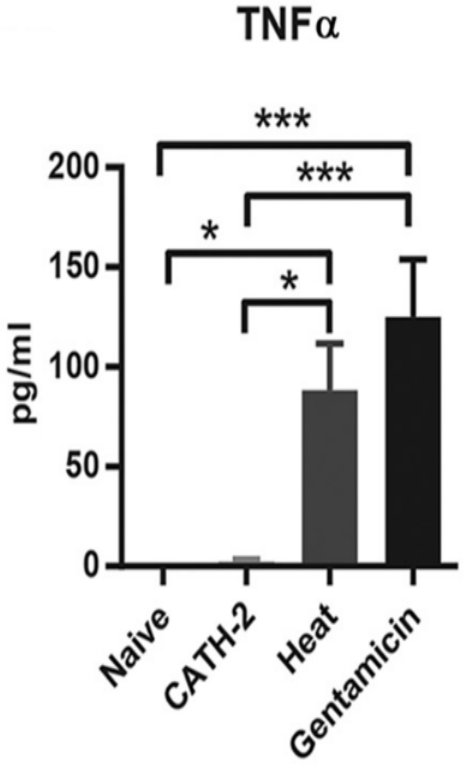
CATH-2 “silently” kills *P. aeruginosa in vitro* and *in vivo*



J774A.1

2h incubation



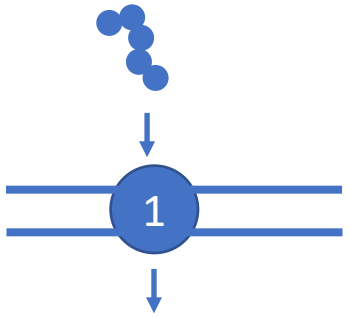


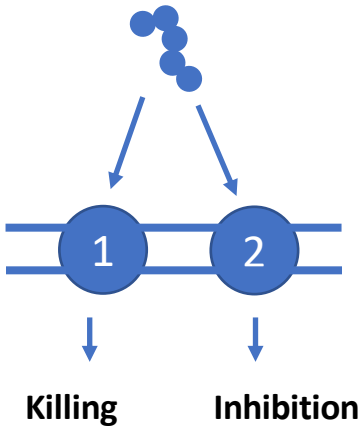
Mouse lung

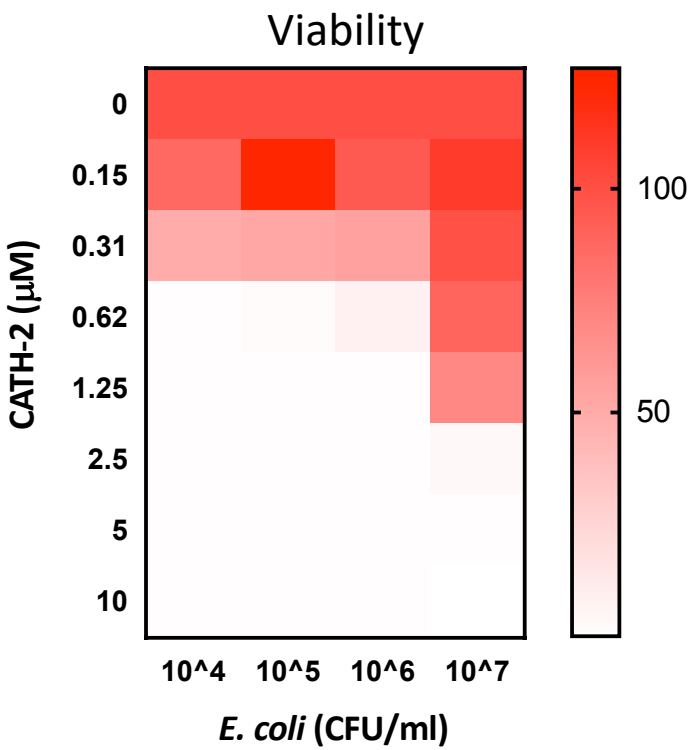
6h after instillation

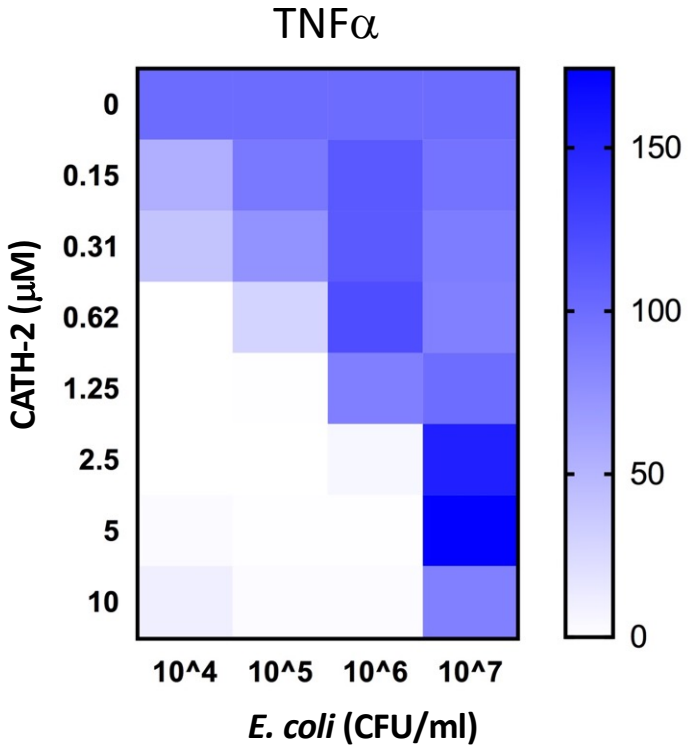
Coorens, Banachewski, et. al. 2017

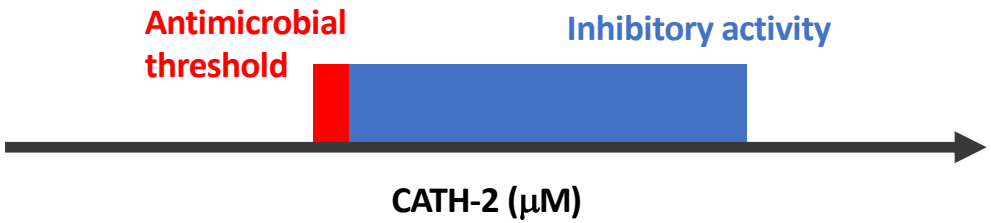
Antimicrobial activity vs. inhibition of macrophage activation





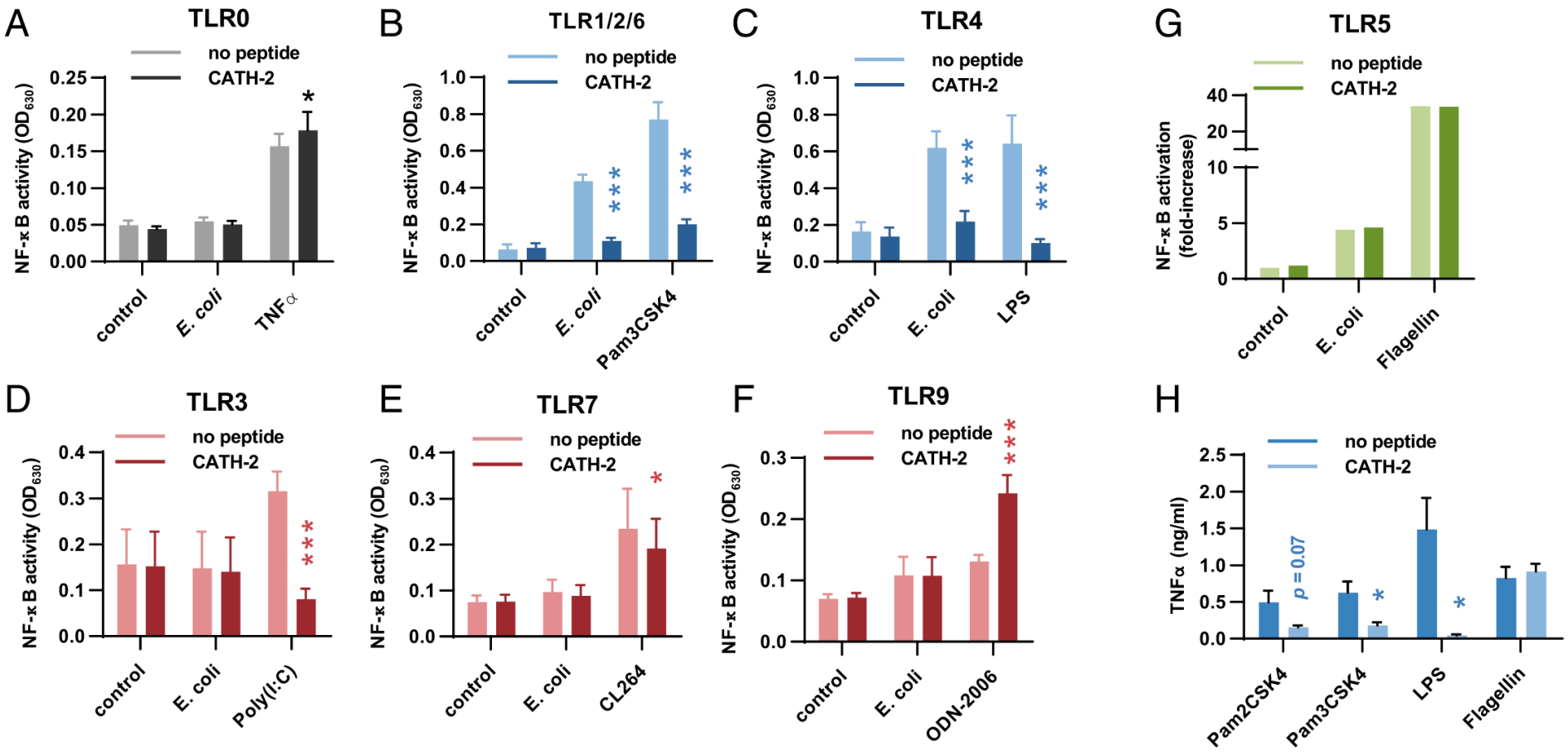






Coorens et al., 2017

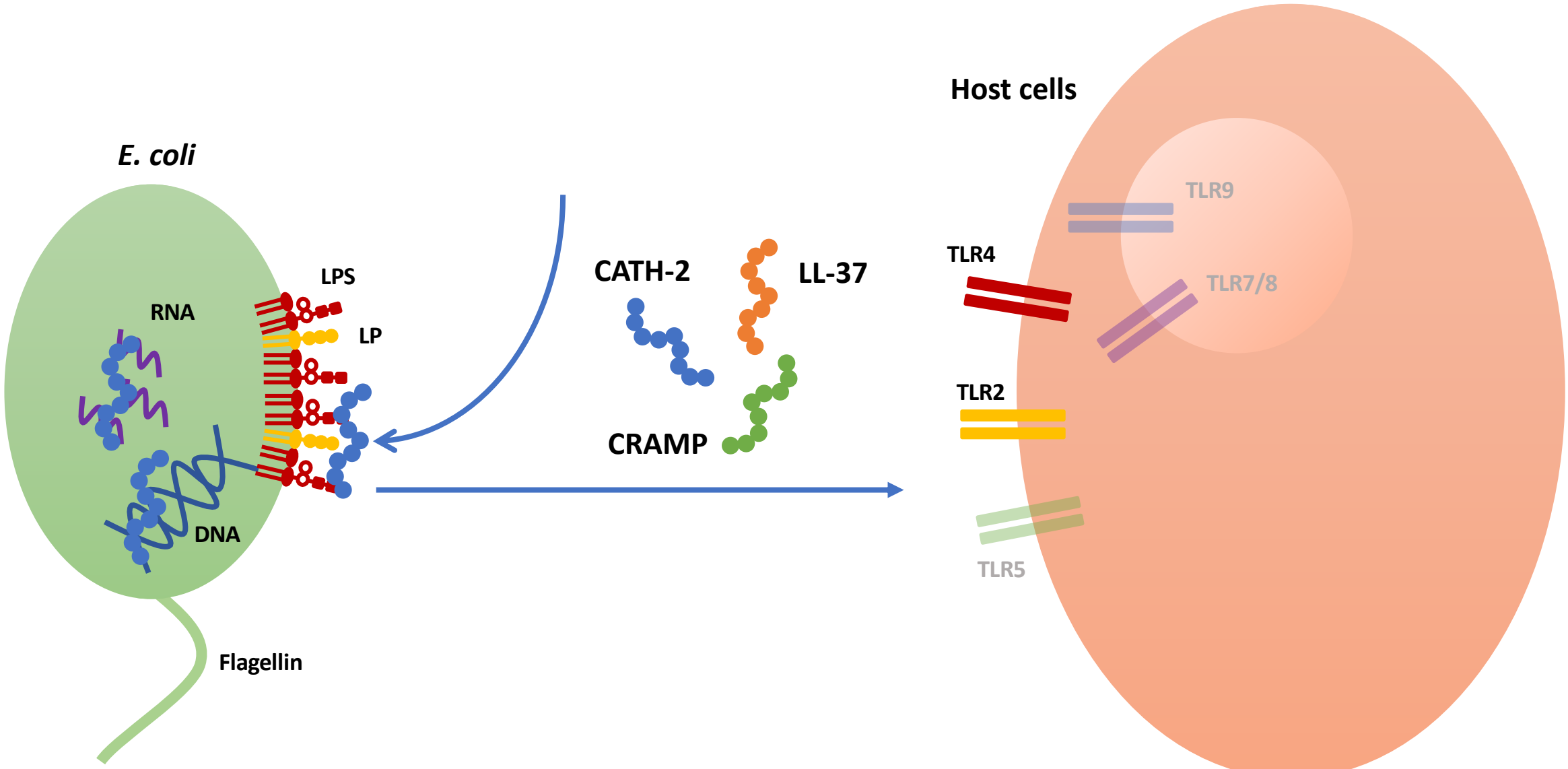
CATH-2 inhibits *E. coli*-induced TLR2 and TLR4 activation



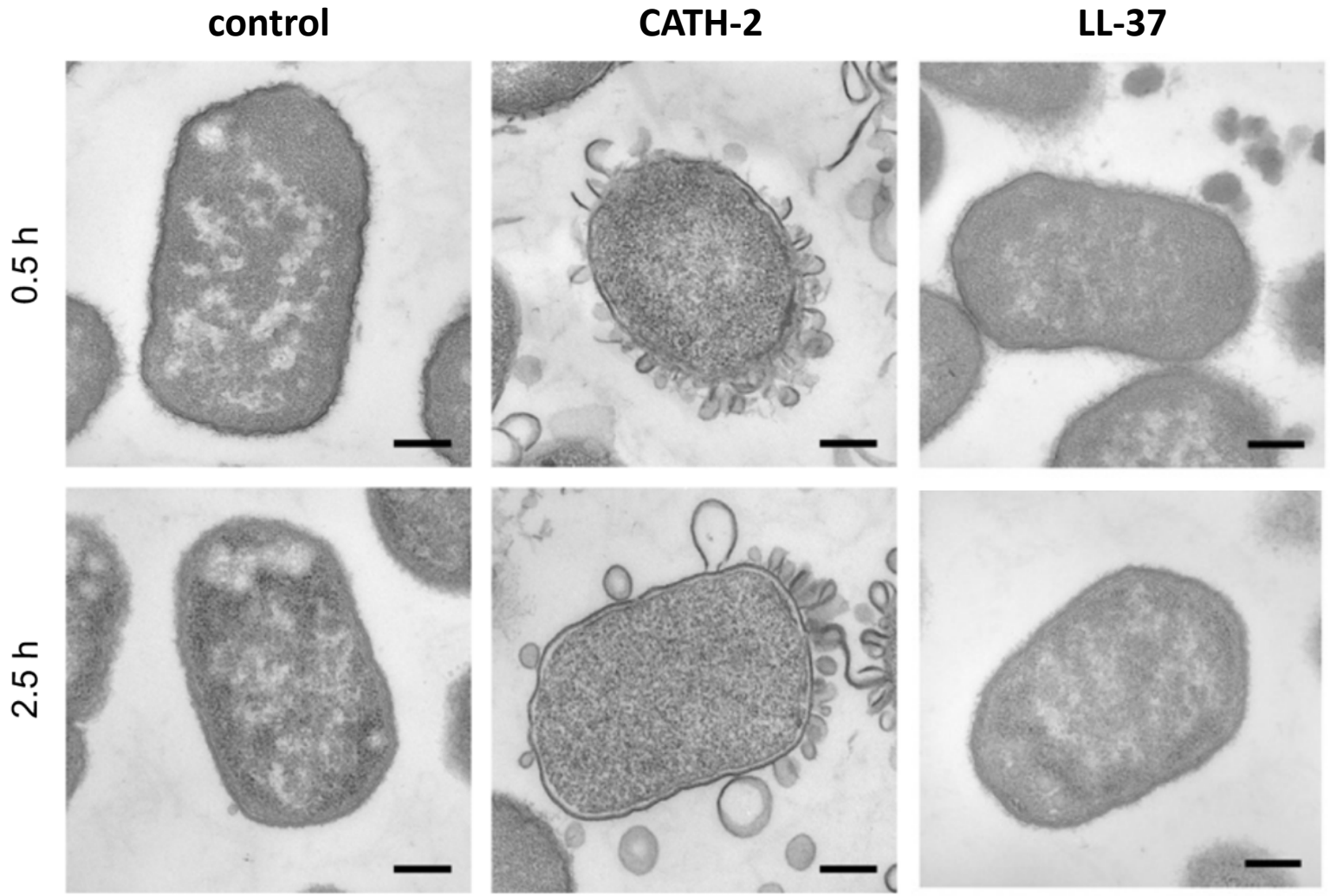
HEK-TLR cells

H: J774.A1 cells

Coorens et al., 2017


E. coli
LPS
LP
RNA
DNA
Flagellin
CATH-2
LL-37
CRAMP
Host cells
TLR9
TLR4
TLR7/8
TLR2
TLR5

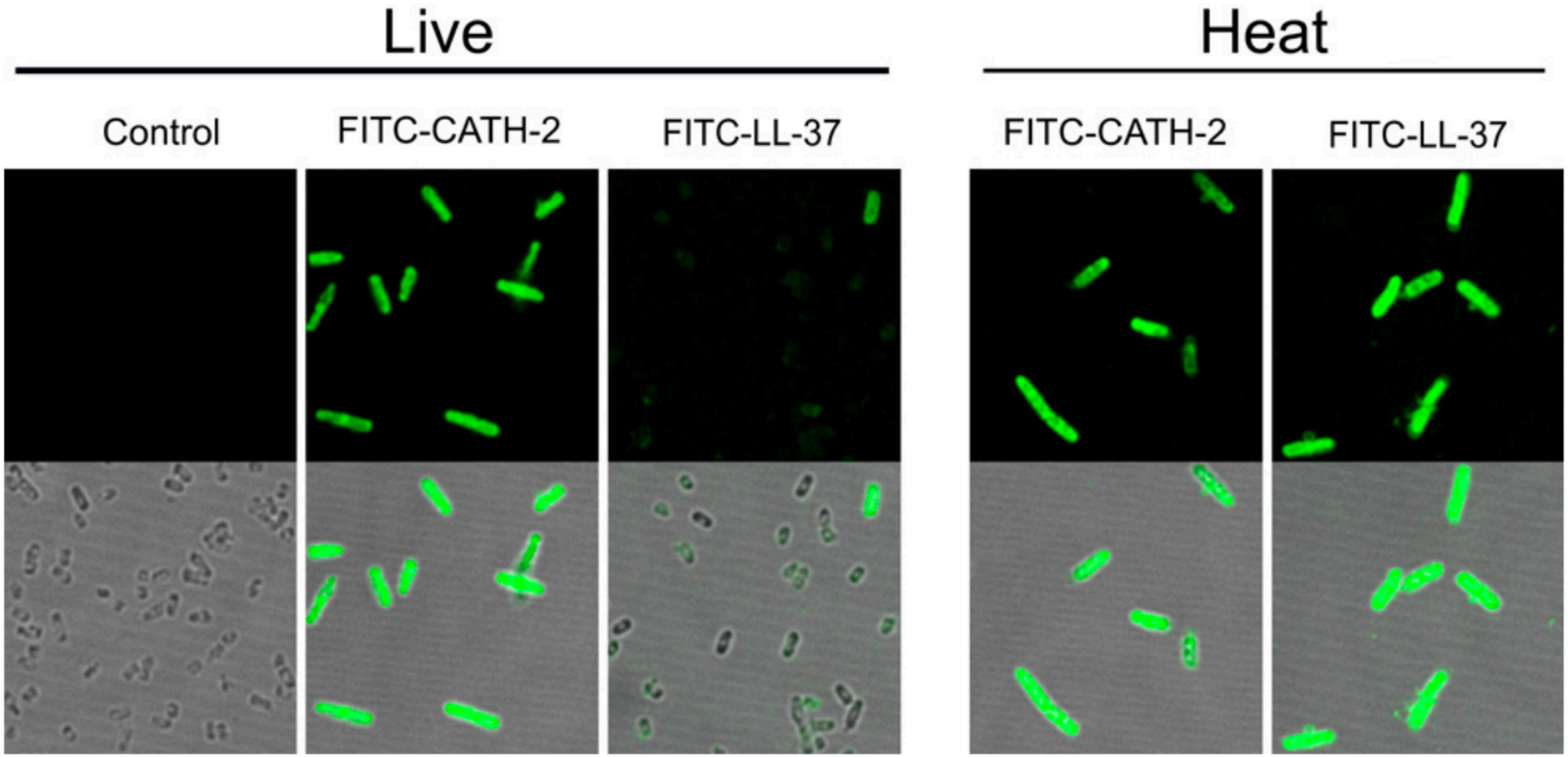
Different effects of CATH-2 and LL-37 on *E. coli* morphology



E. coli O78 (10^8 CFU/ml)
40 µM peptide in DMEM

Coorens et al., 2017

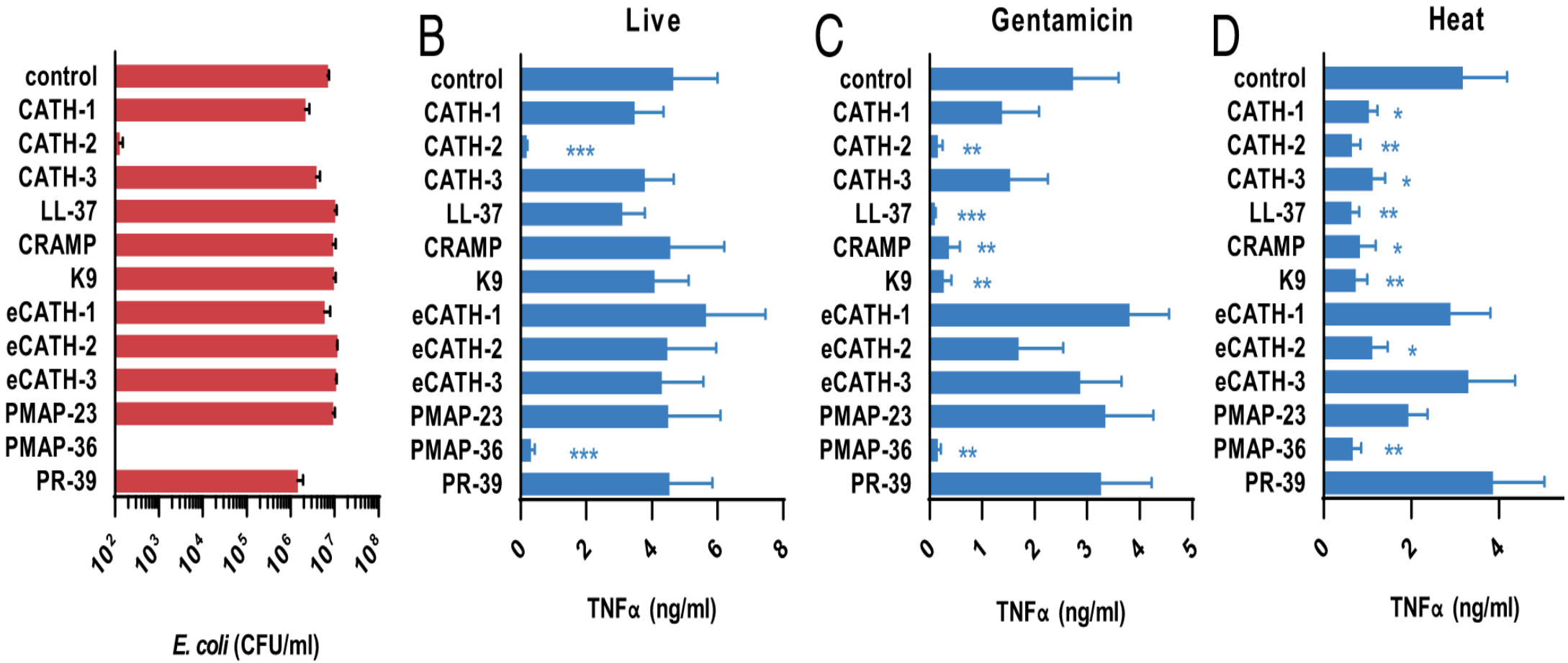
Bacterial viability affects interaction with cathelicidins



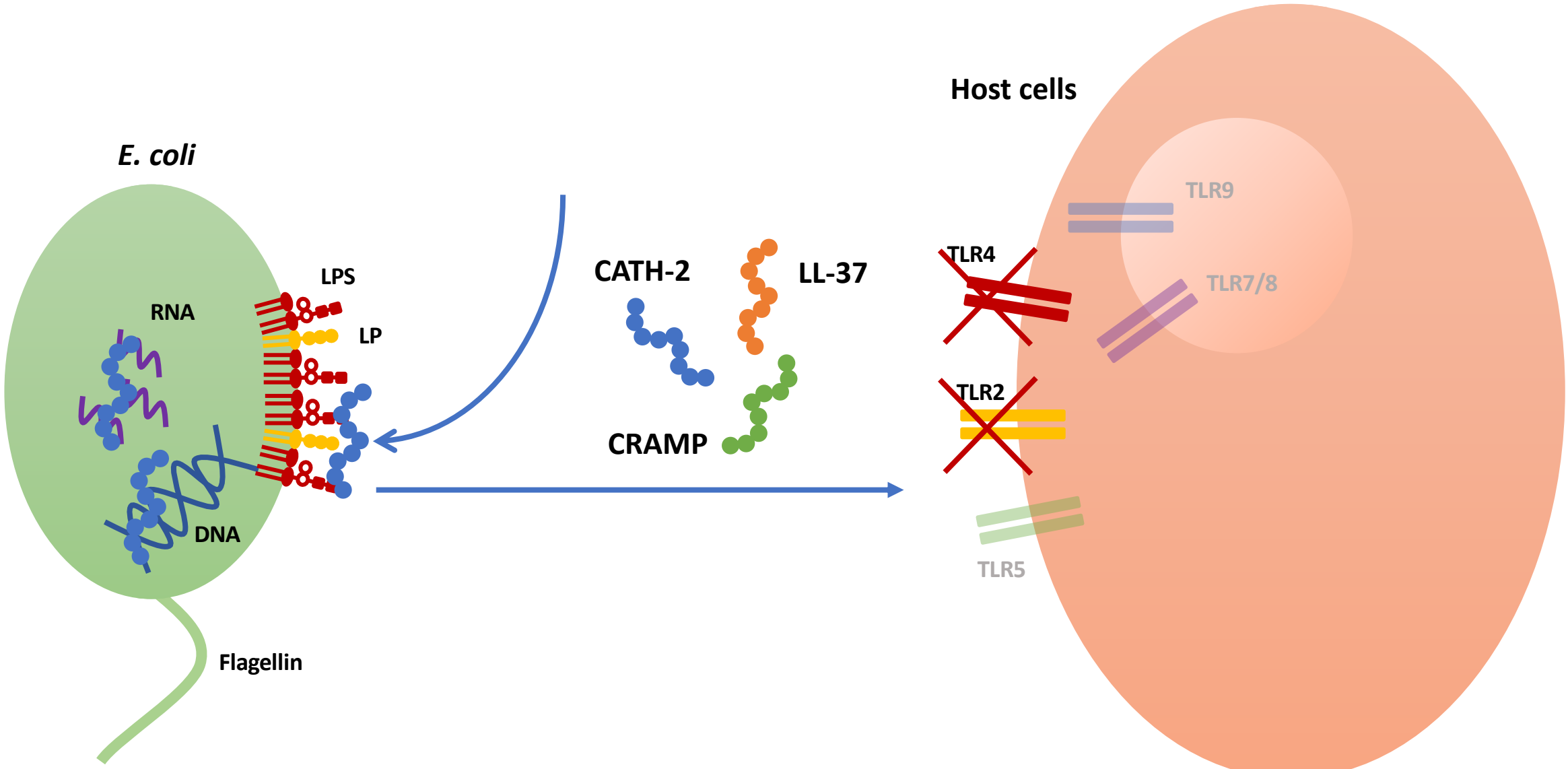
Live or heat-killed *E. Coli* O78 (10^6 CFU/ml); 10 µM FITC–CATH-2 or FITC–LL-37;
2 h in DMEM + 10% FCS

Coorens et al., 2017

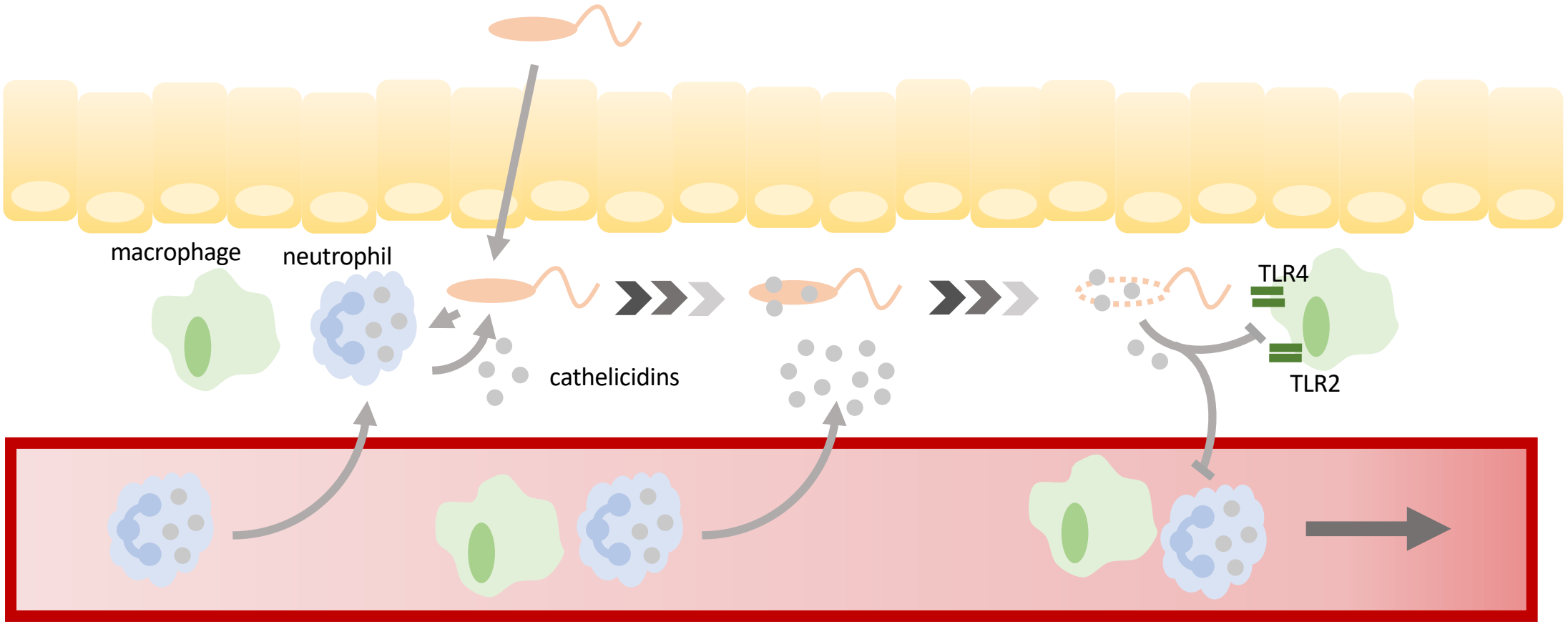
Inhibition of macrophage activation depends on bacterial viability



Coorens et al., 2017


Host cells
E. coli
LPS
LP
RNA
DNA
Flagellin
CATH-2
LL-37
CRAMP
TLR4
TLR9
TLR7/8
TLR2
TLR5

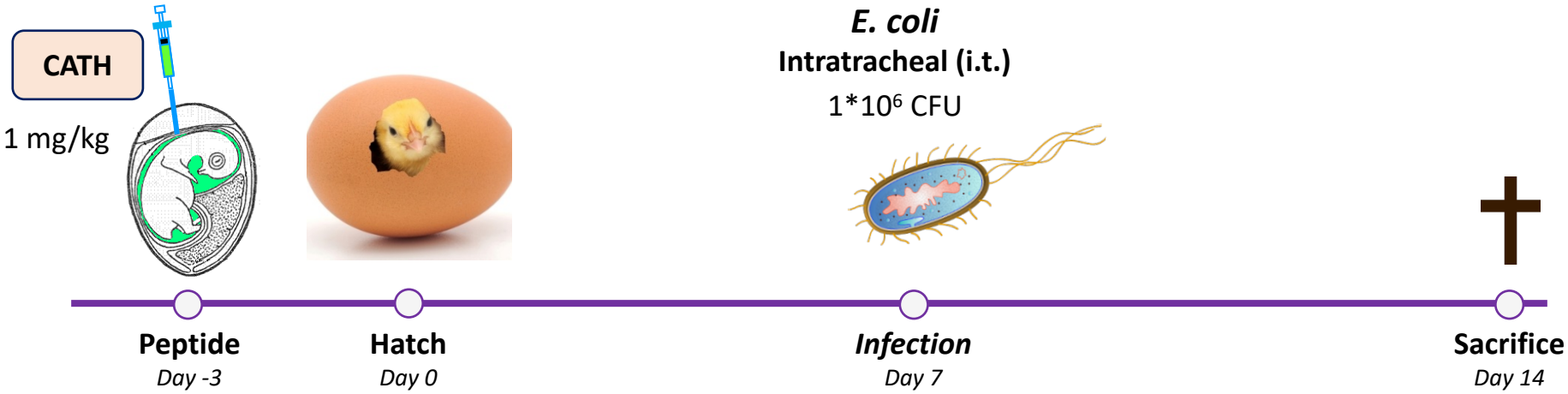
Cathelicidin-mediated “silent killing”

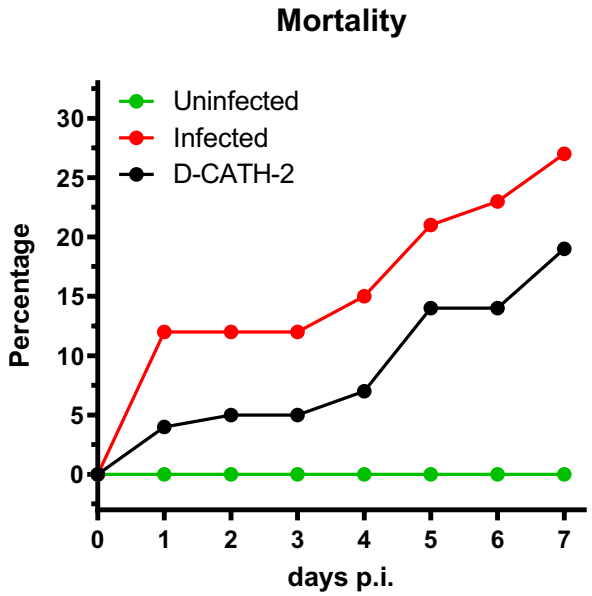


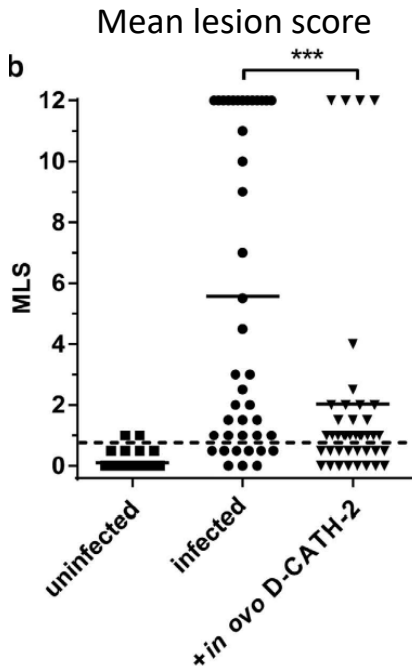
Prophylactic potency of HDPs and derivatives

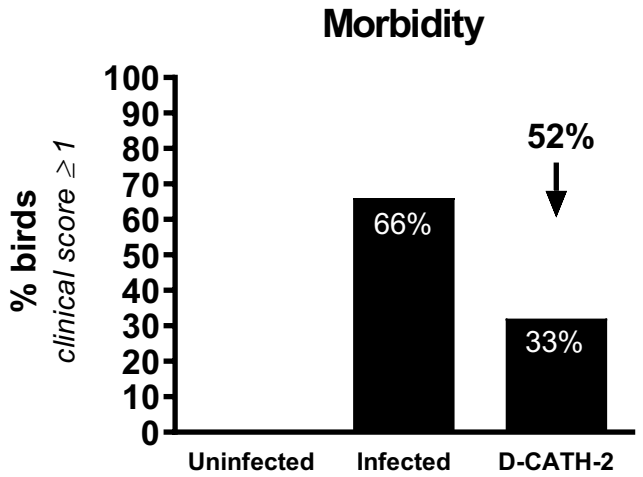
In ovo administration (chicken embryos)

In ovo D-CATH-2 administration protects from colibacillosis



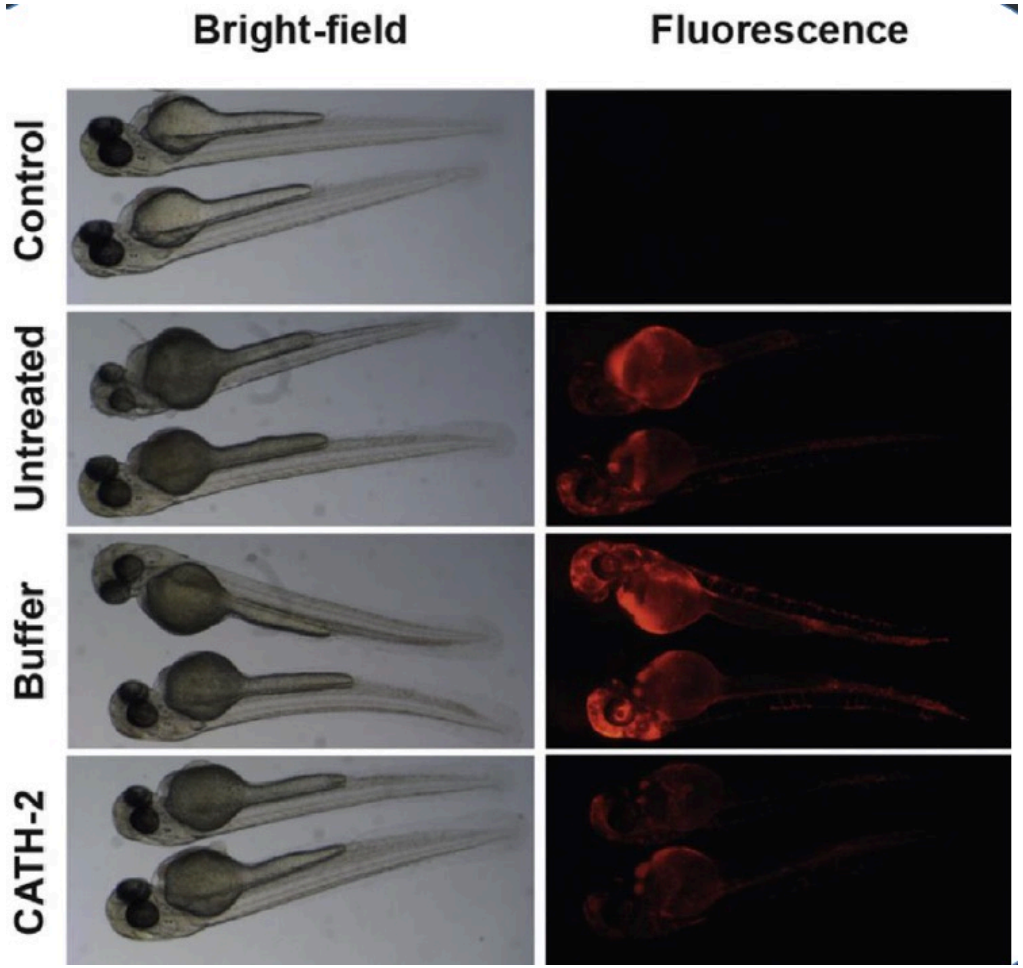






Cuperus, *et a.l.* 2016

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

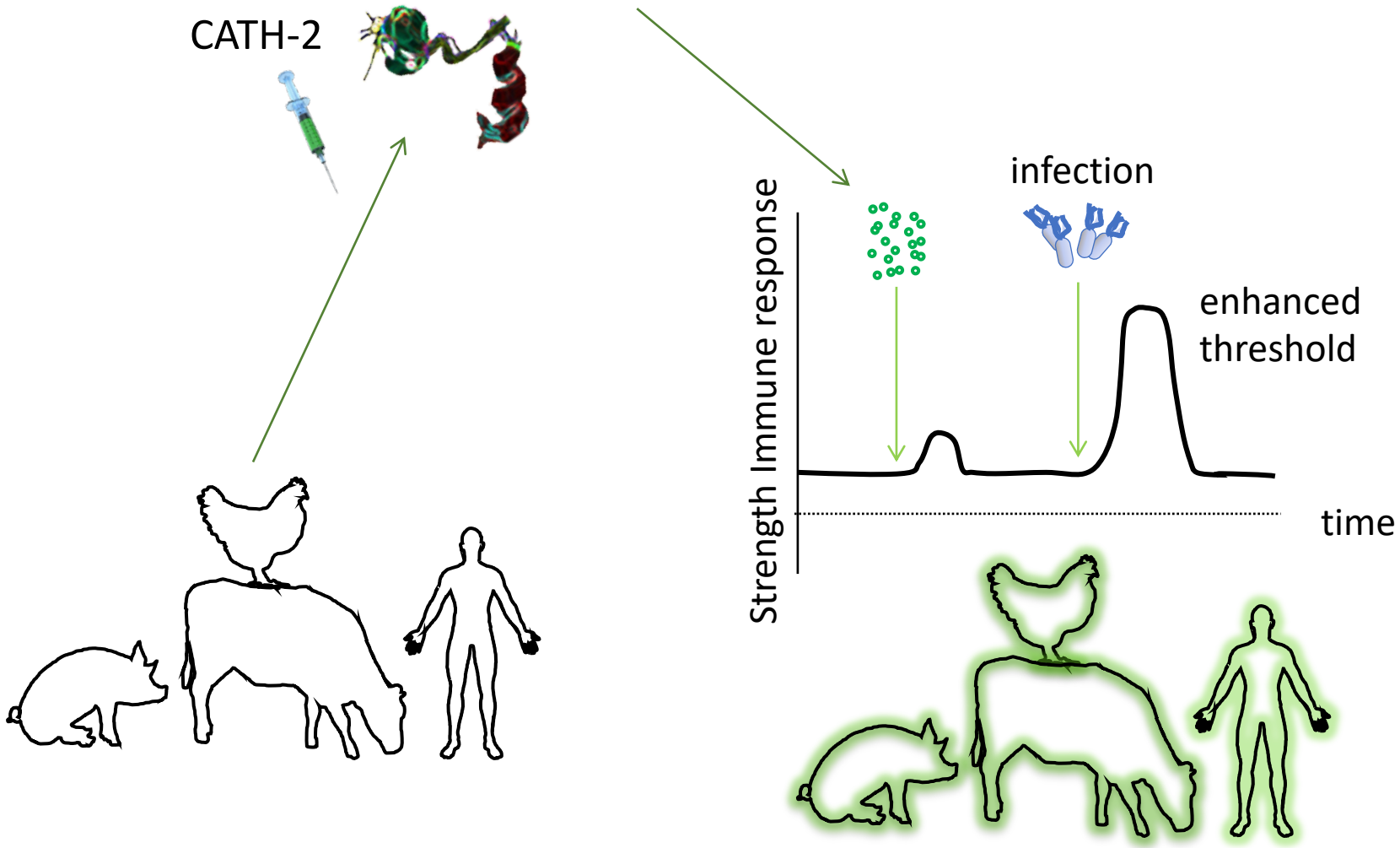
Schneider et al. (2016c)

D-CATH-2 via embryonic route of administration

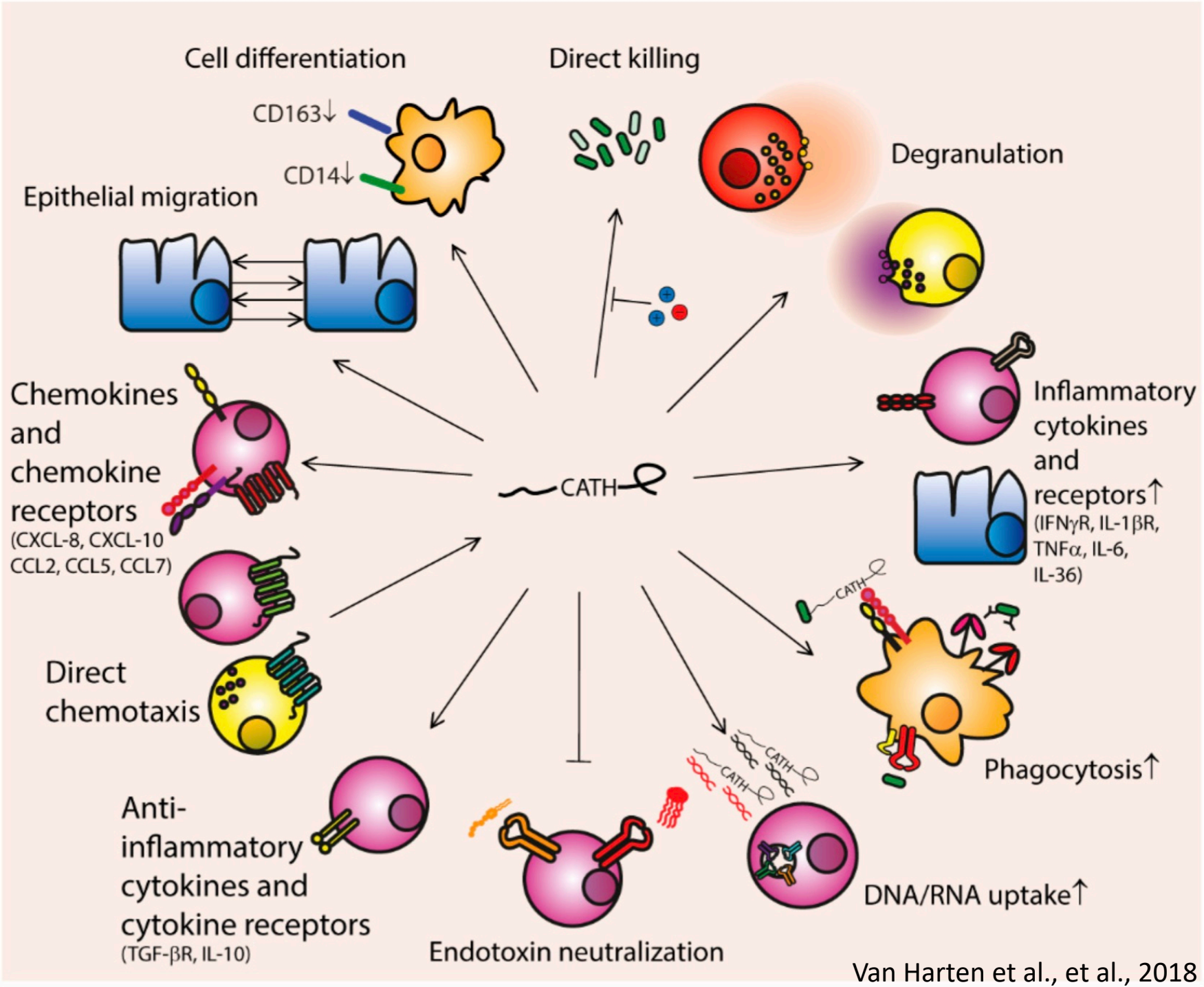
	Target species	Challenge	Result
  Albert van Dijk & Tryntsje Cuperus	**Chicken** *3 day pre-hatch*	***E. coli (i.t.)*** *7 days post hatch*	Mortality 30% Morbidity 52% Bacterial load 93% ↓
 	**Chicken** *3 day pre-hatch*	***S. enteriditis (s.c.)*** *3 days post hatch*	Mortality 50% Morbidity 67% ↓
Viktoria Schneider 	**Zebrafish** *0.2 - 1.5 hpf*	***S. enteriditis*** *28 hpf*	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!**

Peptide-induced innate immune memory?

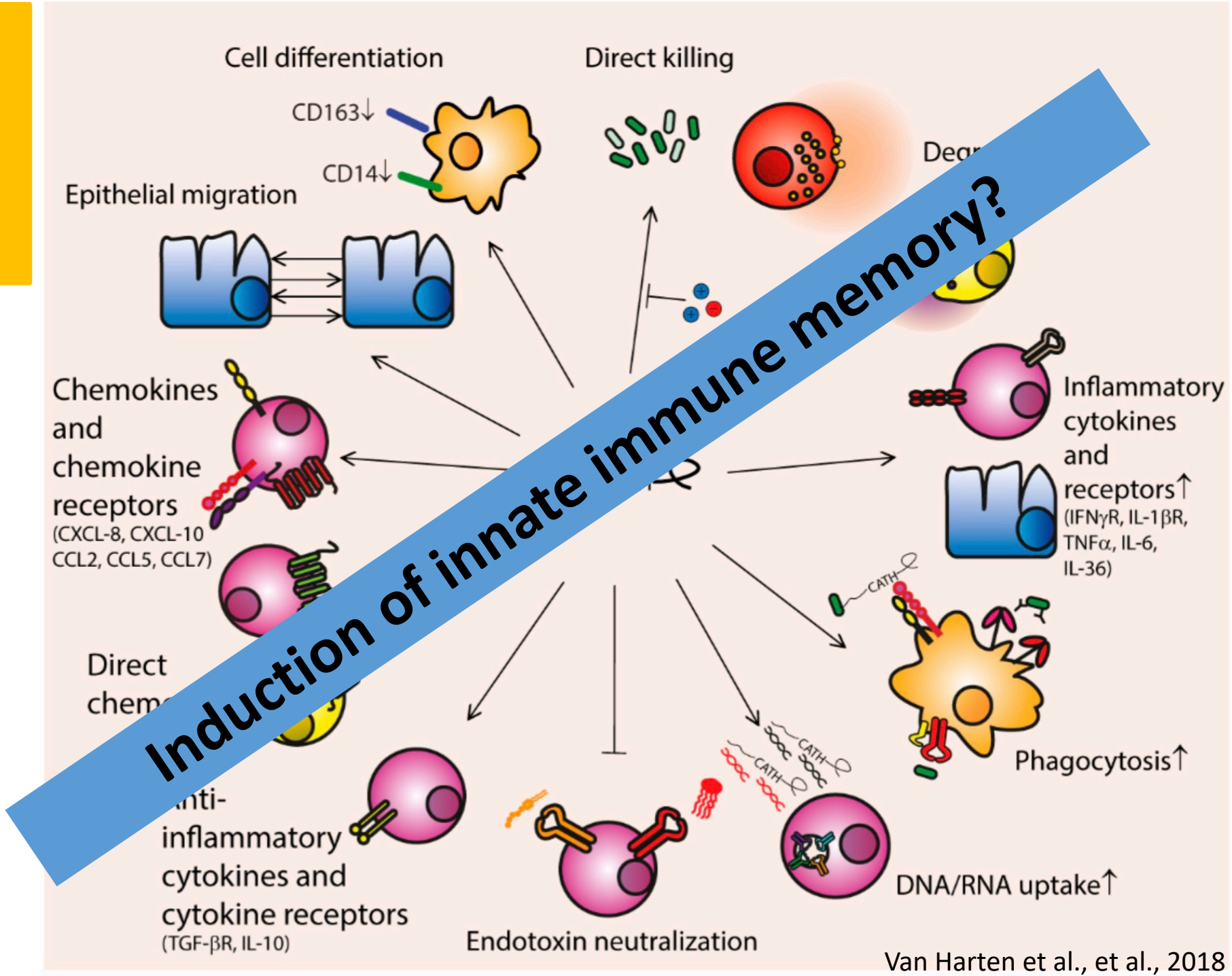


Properties of cathelicidins



Van Harten et al., et al., 2018

Properties of cathelicidins



Van Harten et al., et al., 2018

Applications of cathelicidin-derived peptides

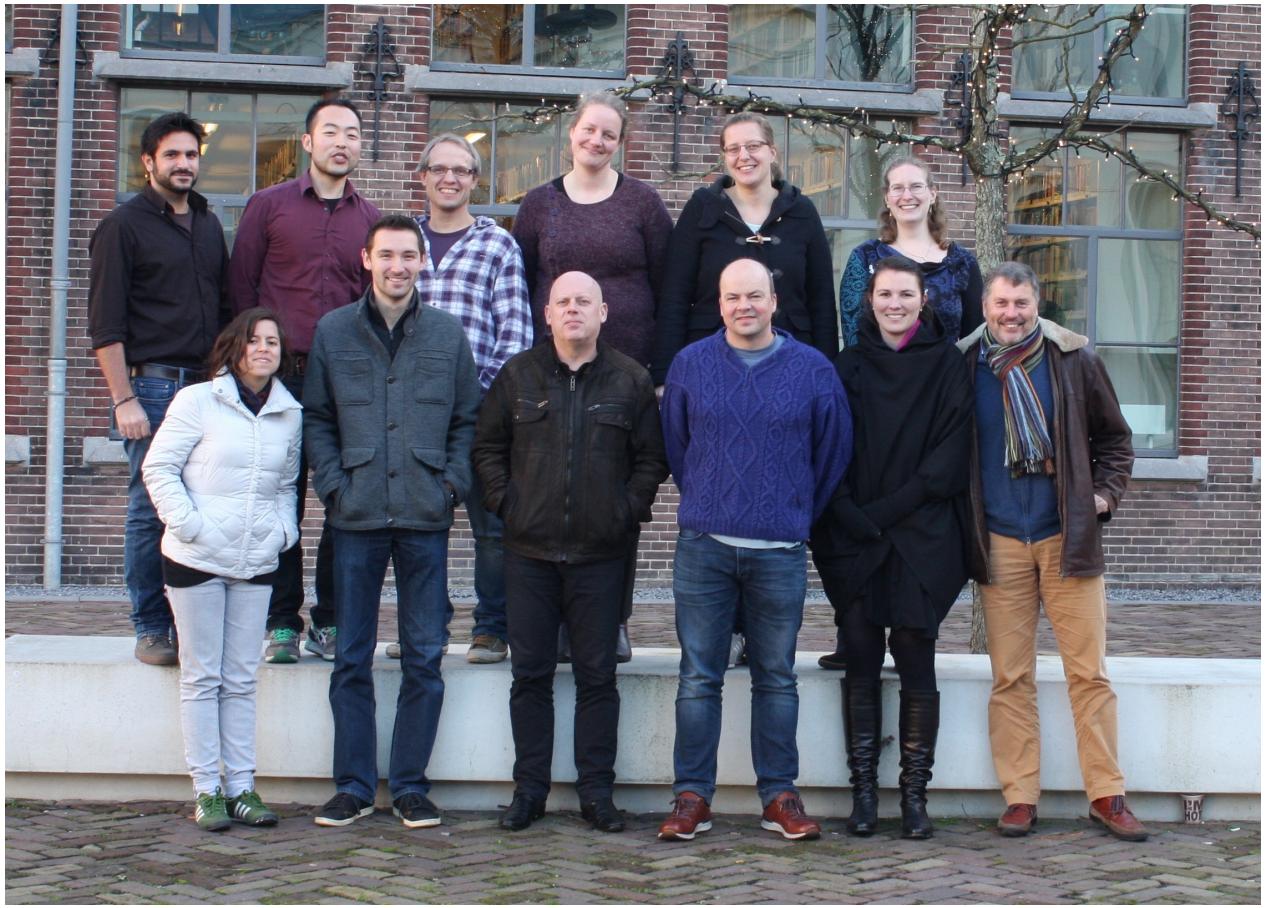
Therapeutic use:

- Direct antimicrobial activity (not systemic)
- Indirect as adjunct to antibiotics

Prophylactic use:

- Immunomodulation *in ovo*
- Postnatal immunomodulation
- In vaccines

Division Molecular Host Defence



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