

In pursuit of a self-assembling protein decoy to counter Enterotoxigenic *Escherichia coli* F4+ strains causing piglet post-weaning diarrhea

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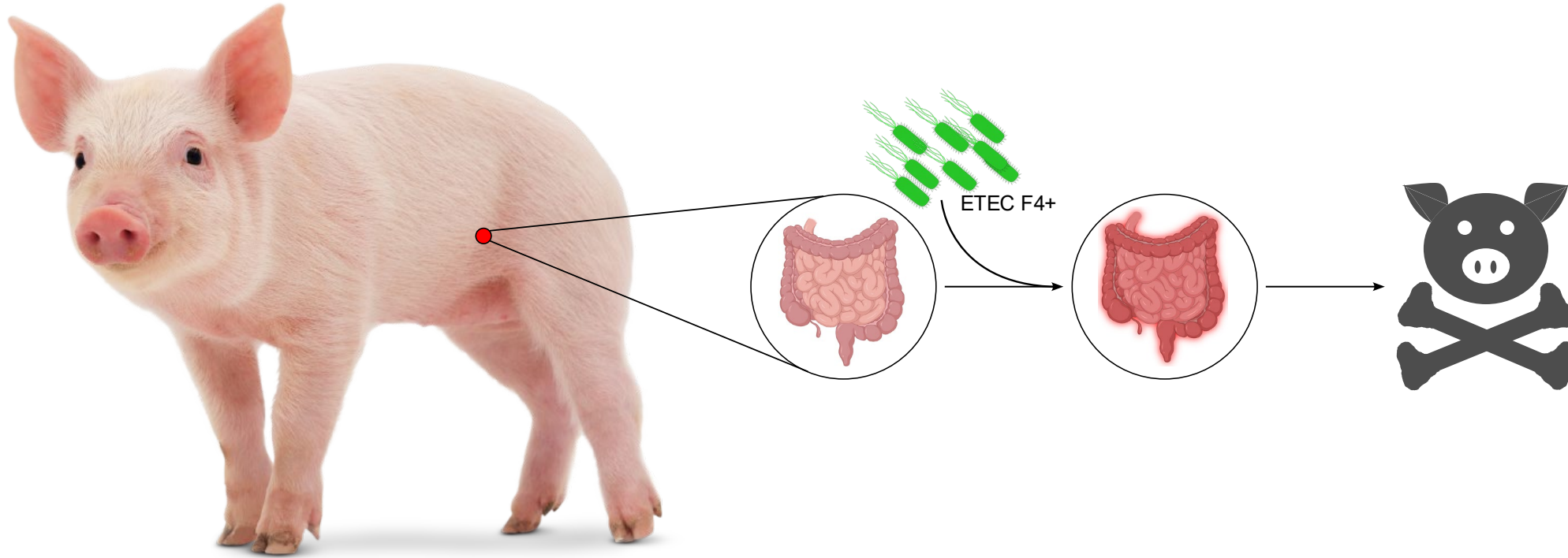


PON
RICERCA
E INNOVAZIONE
2014 - 2020



Unione Europea

Post-Weaning Diarrhea (PWD)



Healthy piglet

Weaned piglet, before
immune system
development

01

Infection

2-5 days after weaning

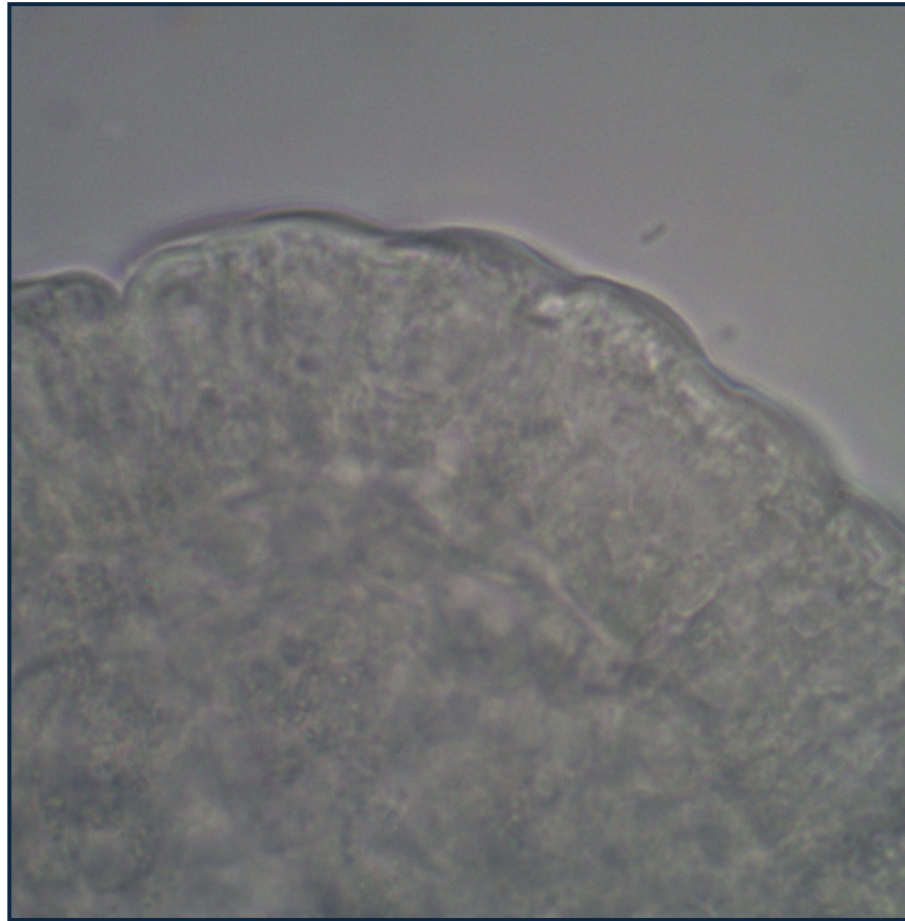
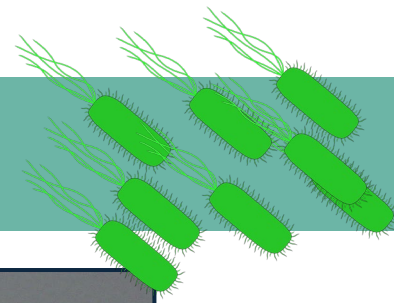
02

Death / weight loss

Few days / weeks after
the infection

03

Enterotoxigenic *E.coli* (ETEC)



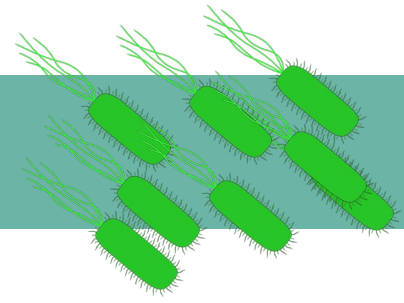
ETEC F4 adhesion at t = 0



ETEC F4 adhesion at t = 60'

Optical microscope image (magnification 100x) of villi surface

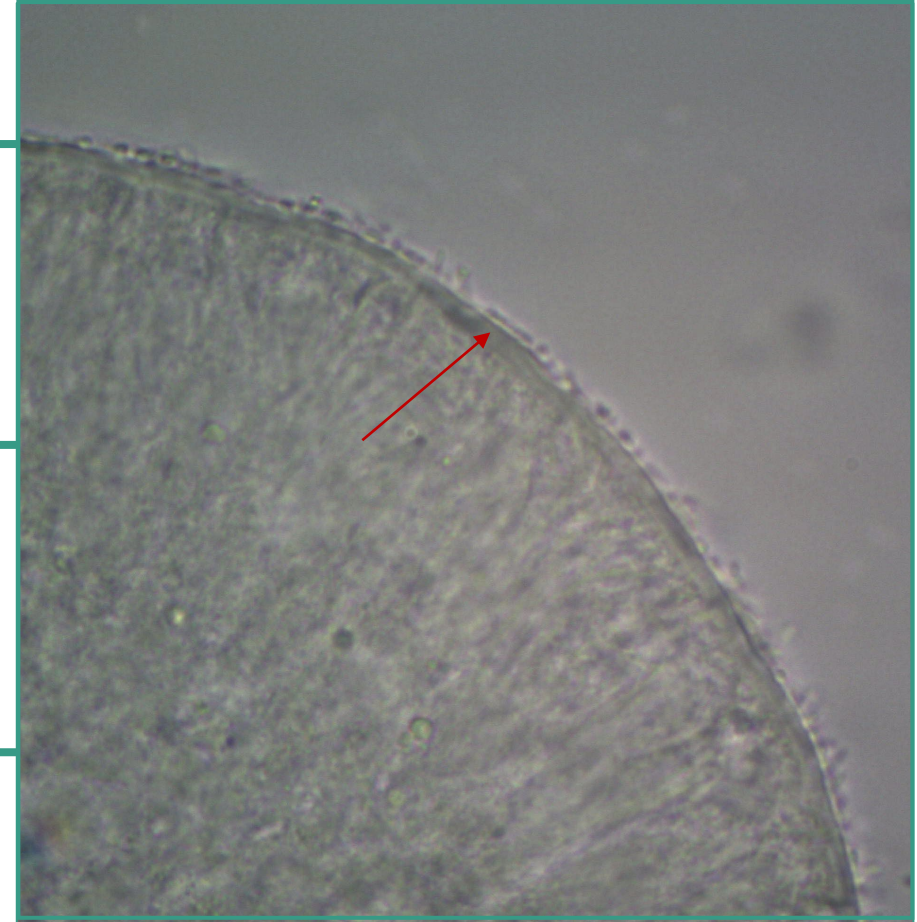
Enterotoxigenic *E.coli* (ETEC)



FaeG component in ETEC F4+ fimbriae adhere to the intestinal surface

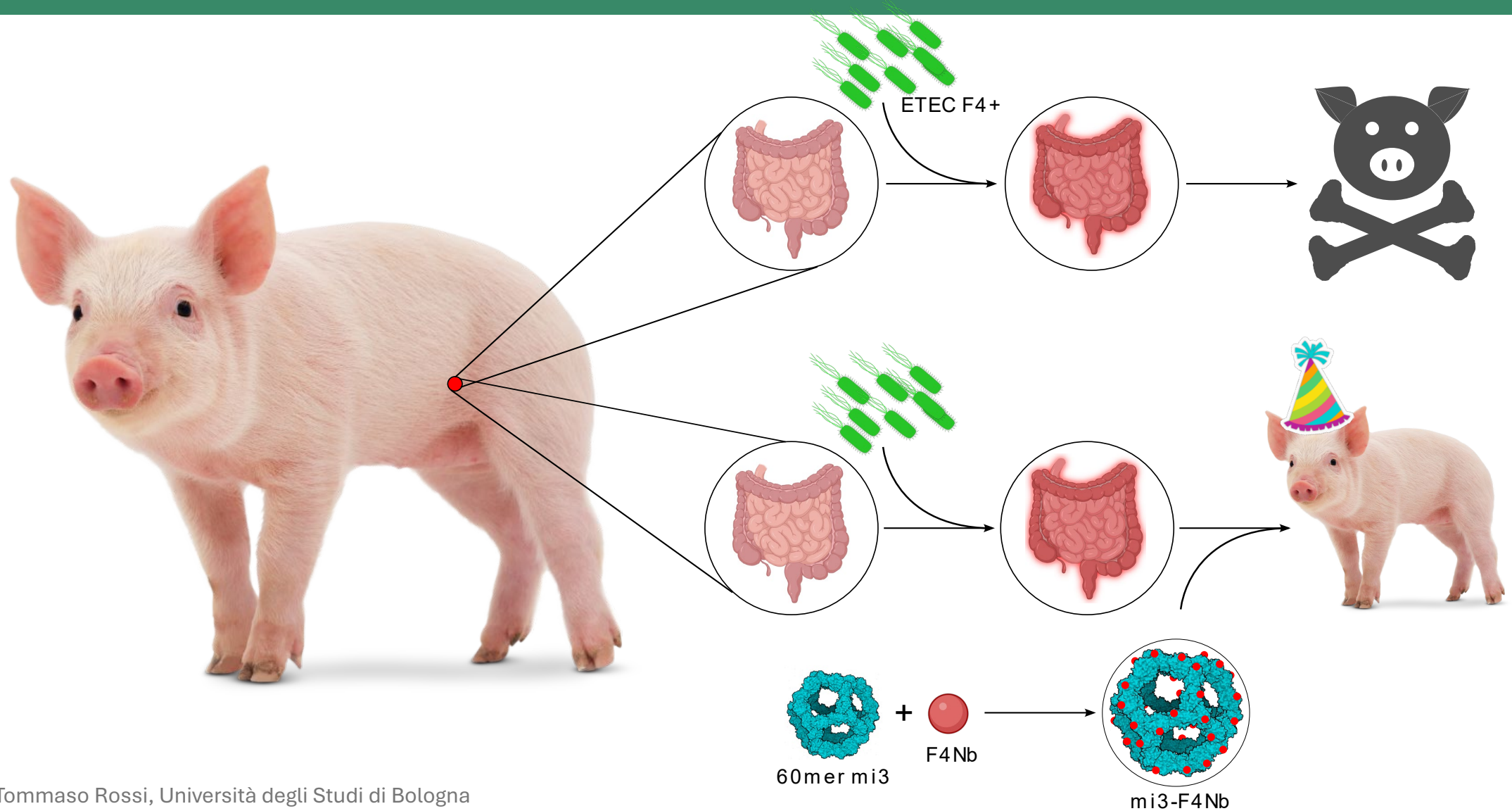
In-vivo incubation time around 2-3 days a.w.

Heat-labile enterotoxin (LT), heat-stable enterotoxins (ST) induce a transient diarrhea

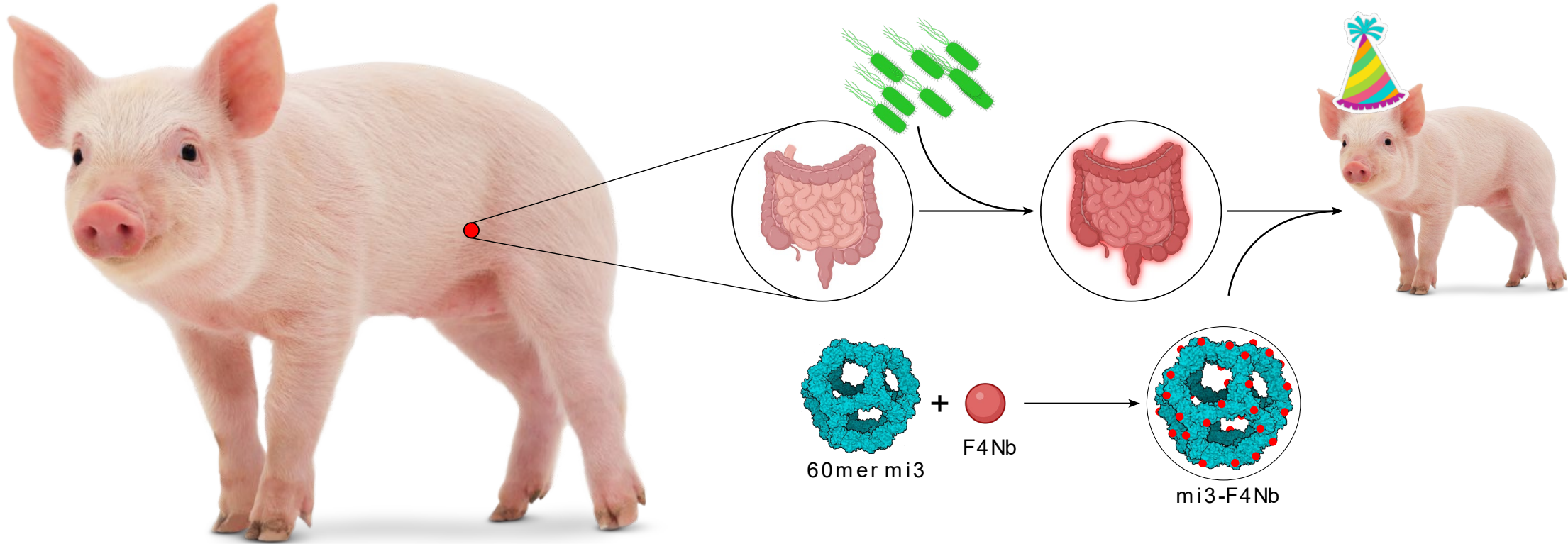


ETEC F4 adhesion at t = 60'

Nanoscaffold to prevent adhesion



Nanoscaffold to prevent adhesion



Healty piglet

Weaned piglet, before
immune sistem
development

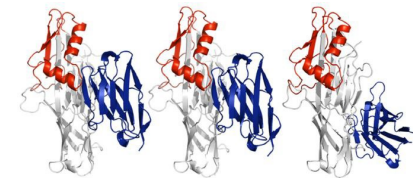
01

Infection

2-5 days after weaning

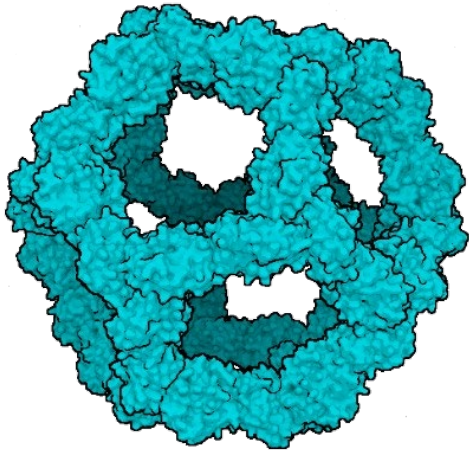
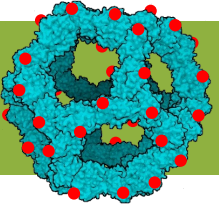
02

VLP/Nbs anti-ETEC



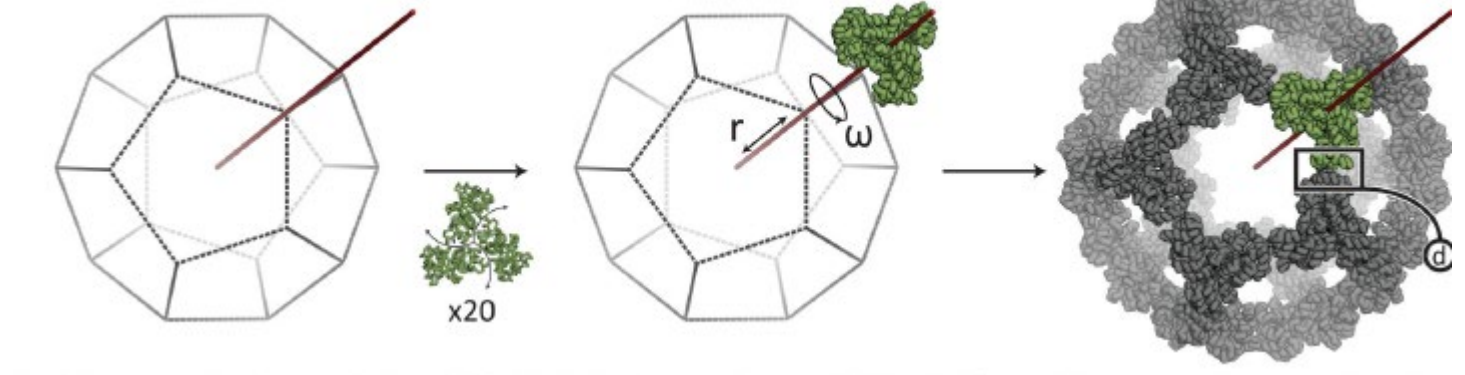
03

VLP/Nbs anti-ETEC



Model of 60-mer mi3 self-assembling protein

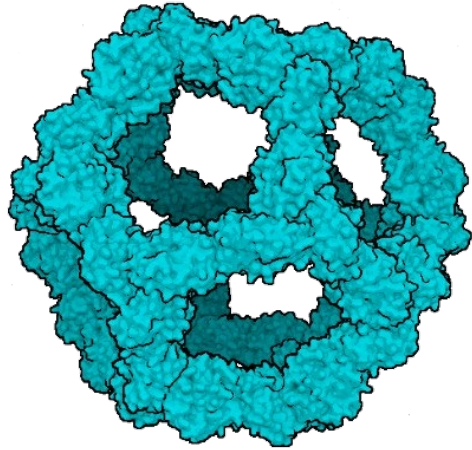
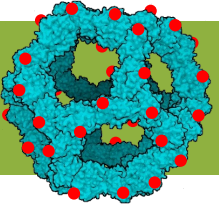
=



(D.Baker et al. 2016)

- ↑ Protein-based nanoscaffold
- ↑ Plug-and-display system
- ↑ Stability

VLP/Nbs anti-ETEC

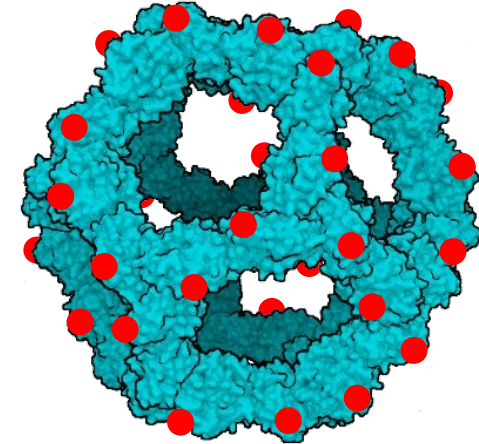


Model of 60-mer mi3 self-assembling protein

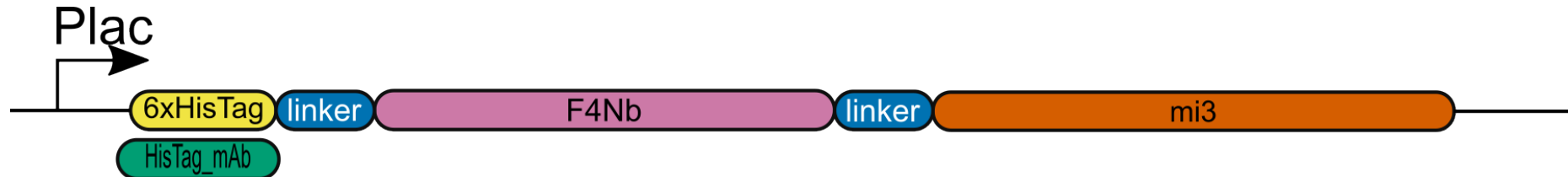


Nanobody
F4Nb

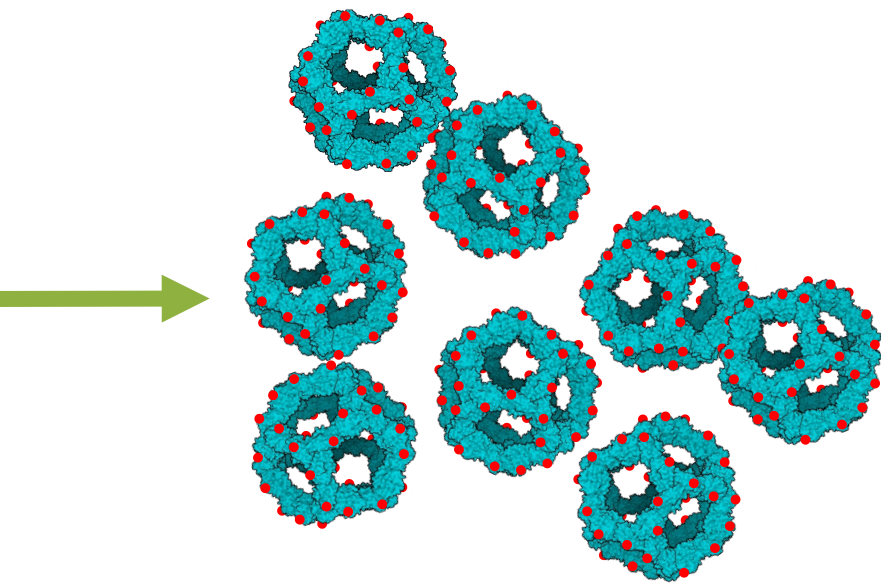
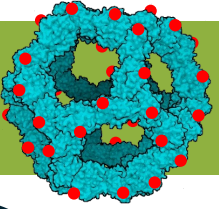
Production in *E.coli* BL21



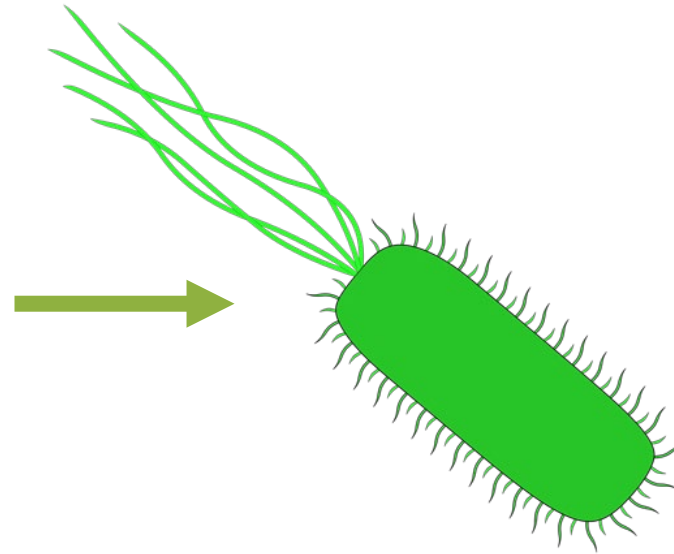
mi3-60xF4Nb +
HisTag



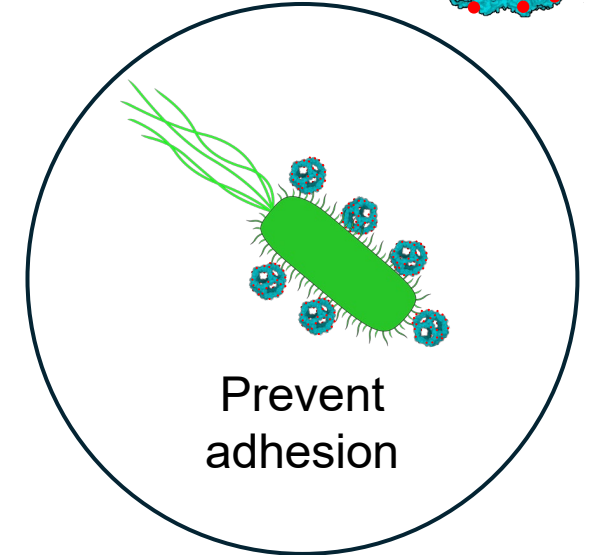
VLP/Nbs anti-ETEC



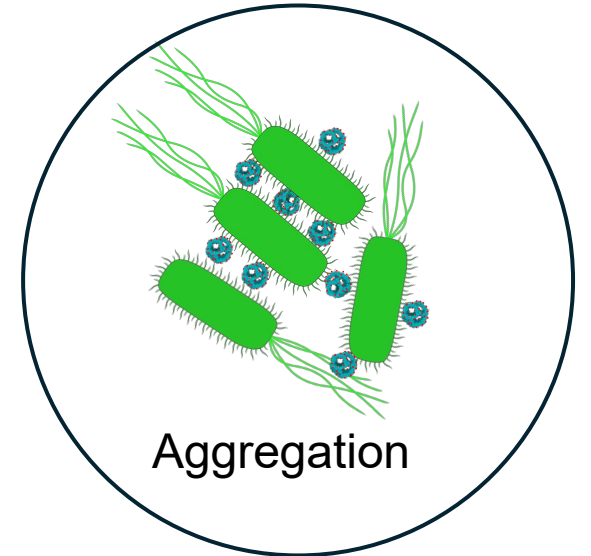
mi3-60x F4Nb



ETEC F4+

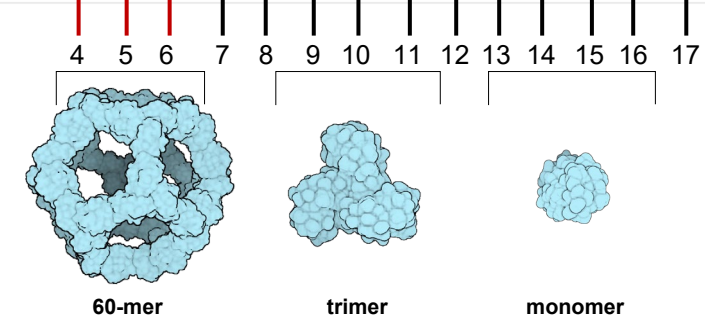
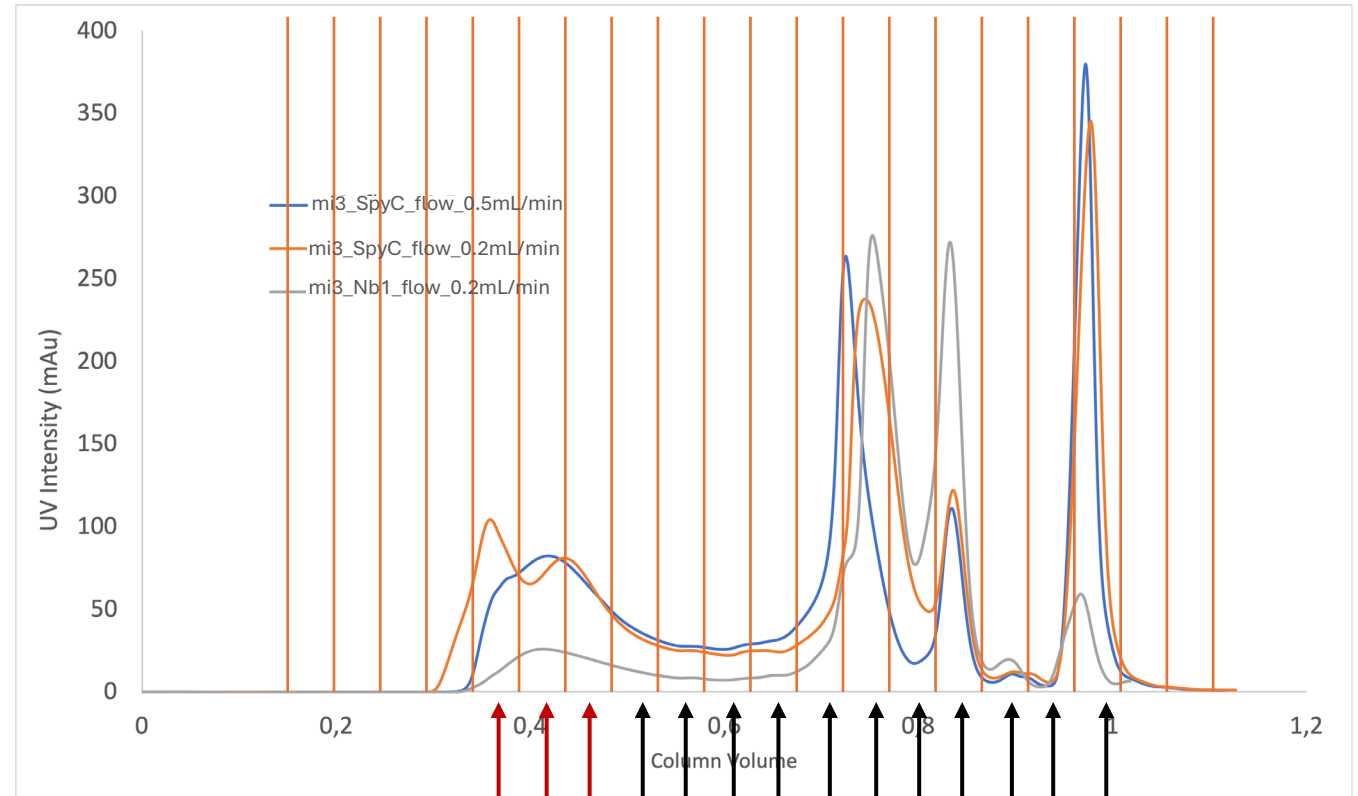
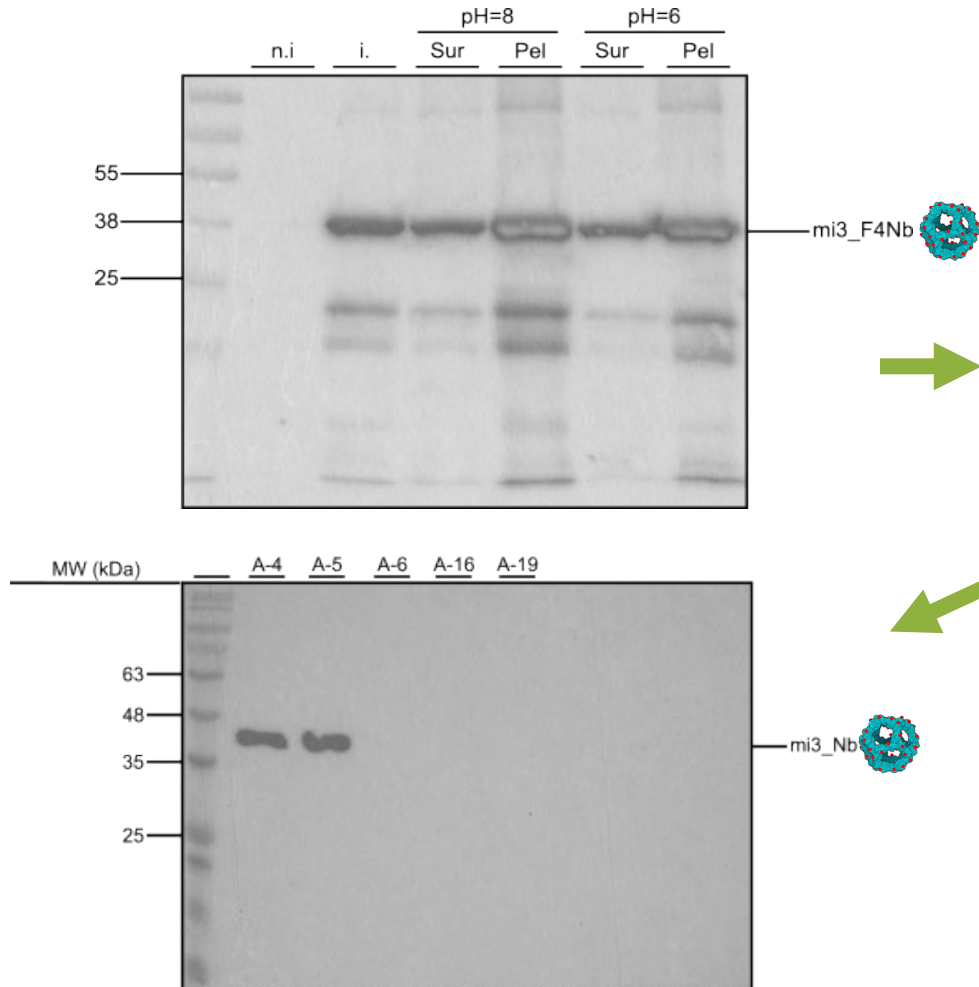
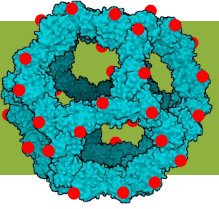


Prevent
adhesion



Aggregation

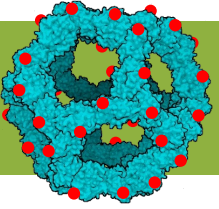
mi3F4Nb Purification in AKTA pure®



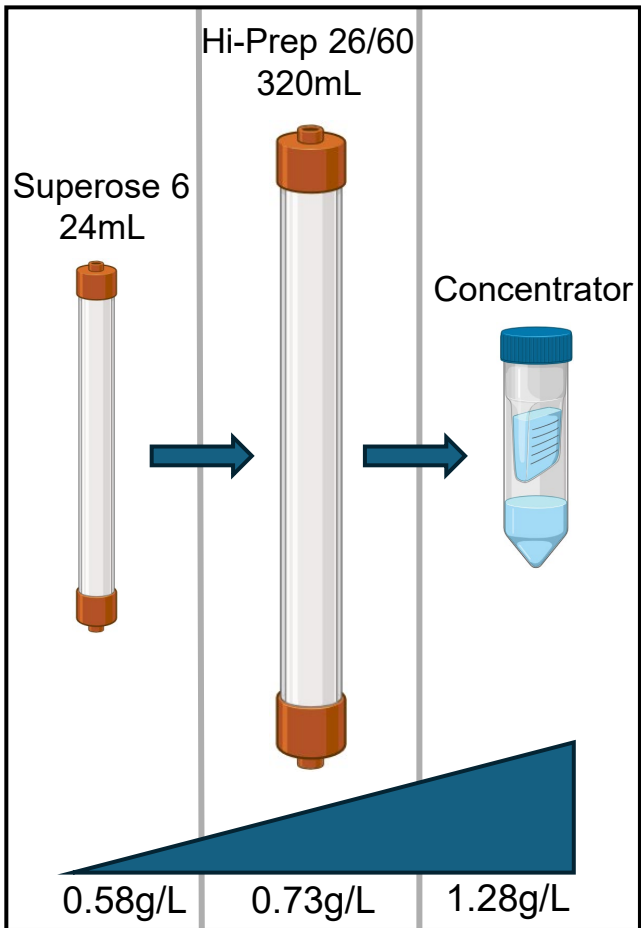
Upper case: Wb expression and purification of mi3Nb at different pH
Lower case: Wb anti-HisTag AKTA fraction A4,A5,A6,A19



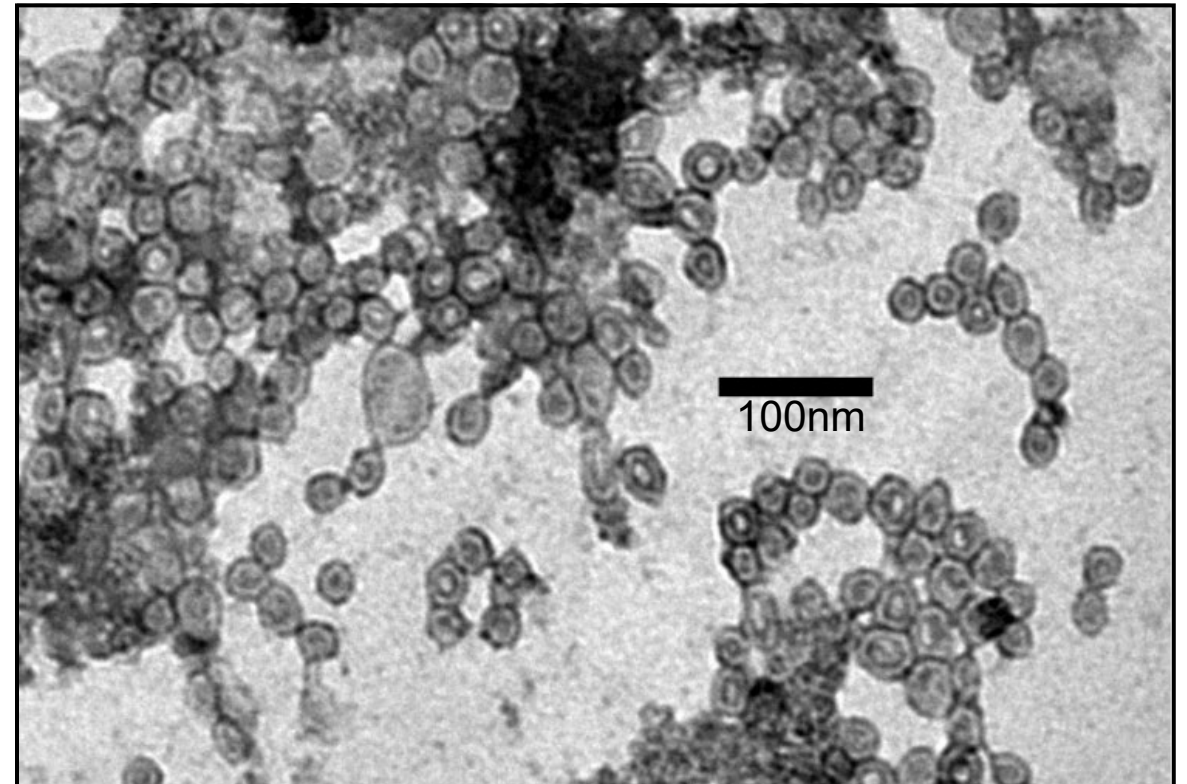
Production and microscopy analysis



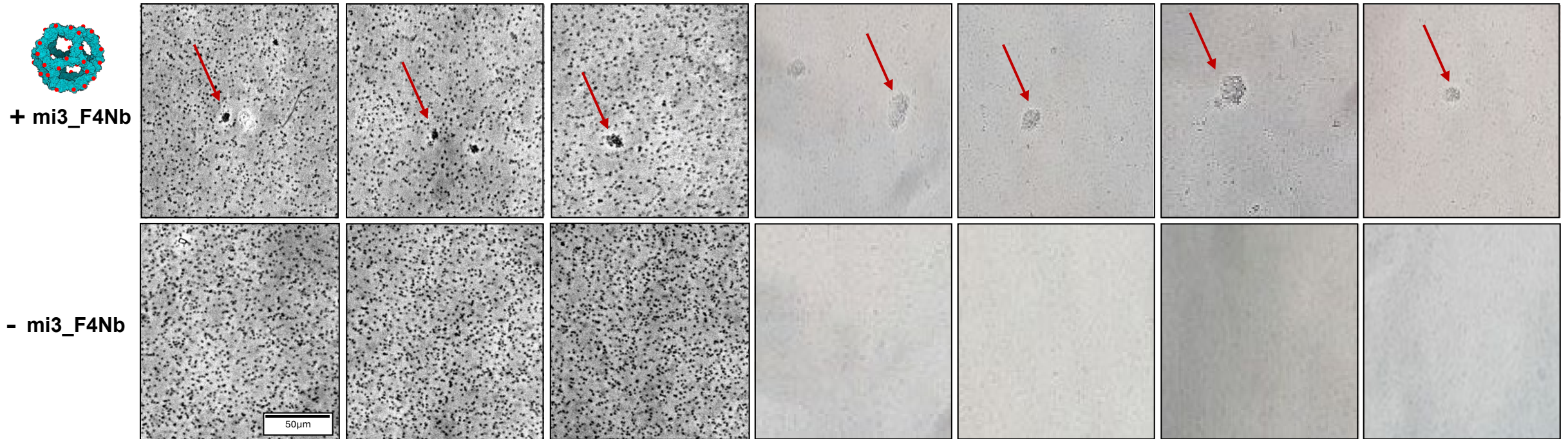
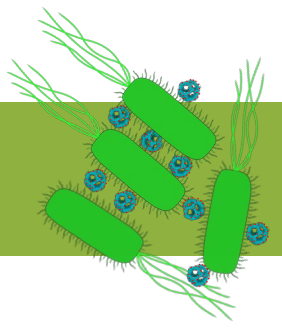
↑ yield mi3-F4Nb 60-mer



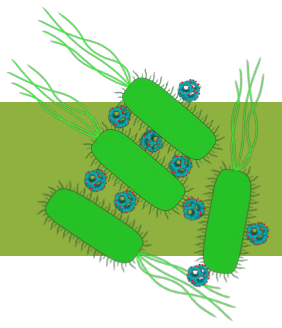
TEM picture of mi3_F4Nb

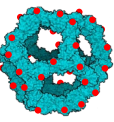


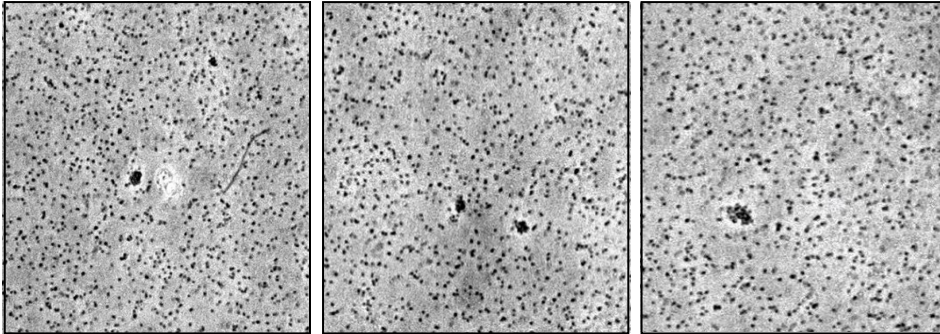
mi3-Nb/ETEC F4+ aggregation assay



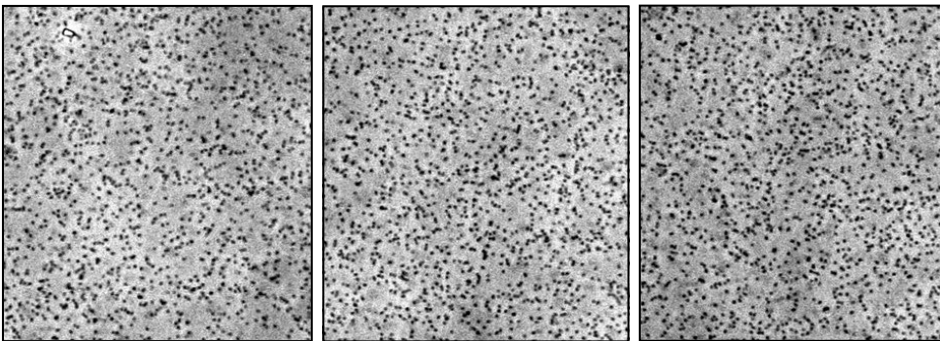
mi3-Nb/ETEC F4+ aggregation assay



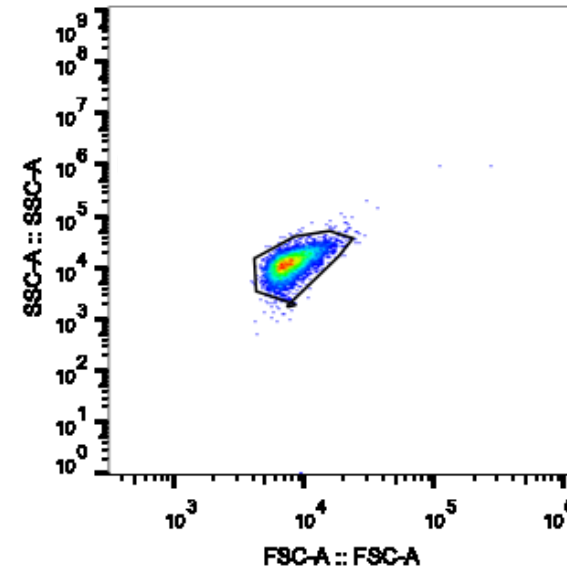

+ mi3_F4Nb



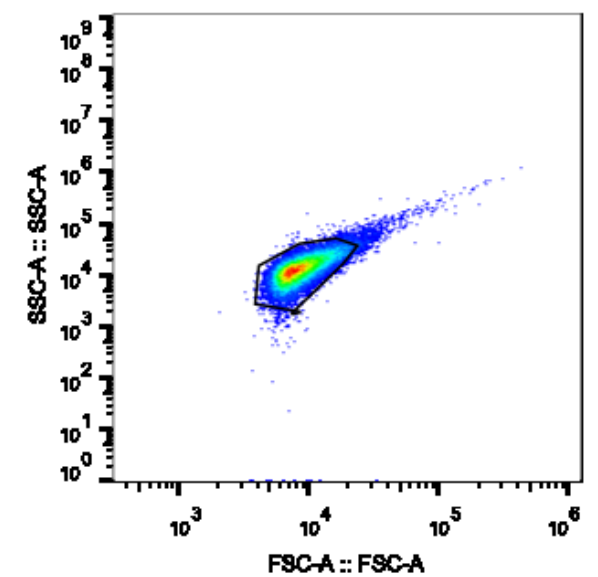
- mi3_F4Nb



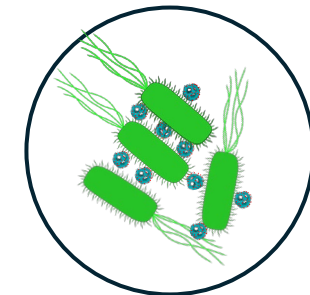
Untreated ETEC



ETEC + mi3



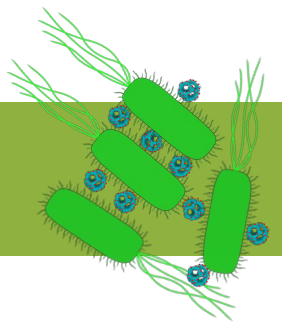
Flow cytometry analysis



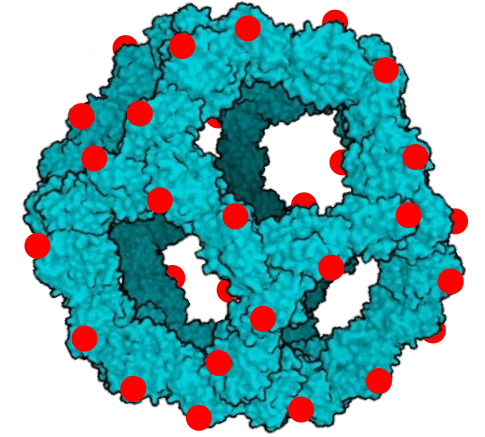
= 5%

Aggregation

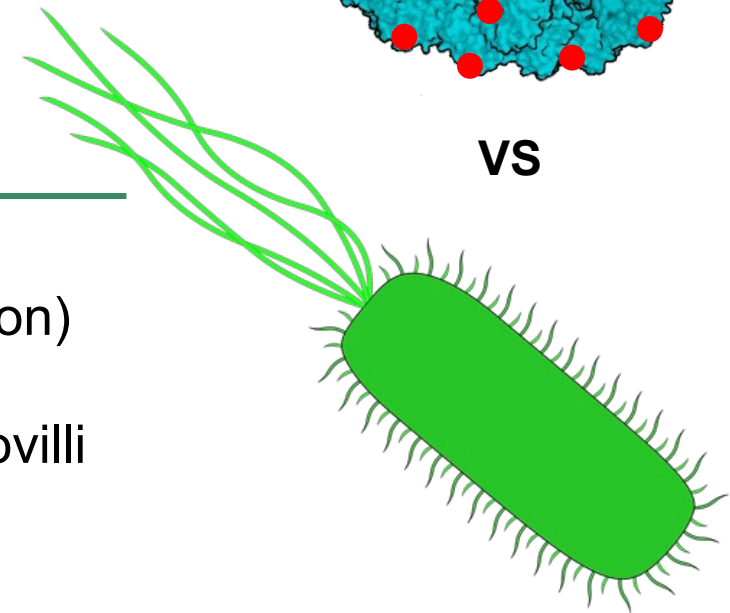
Conclusions and future perspectives



- 1) ↑ mi3 60-mer platform to display nanobodies/small molecules like mi3F4Nb
- 2) ↑ Good protein yield after clarification and concentration
- 3) ↑ confirmed ETEC – mi3_F4Nb interaction



VS



Improve nanoscaffold/bacteria interaction (test other Nbs in fusion)

Test *ex vivo* performance in ETEC F4+ adhesion model of microvilli

Extend the nano-assembly platform to other pathologies

Acknowledgement

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Alena Kaltenbrunner

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Thank you!