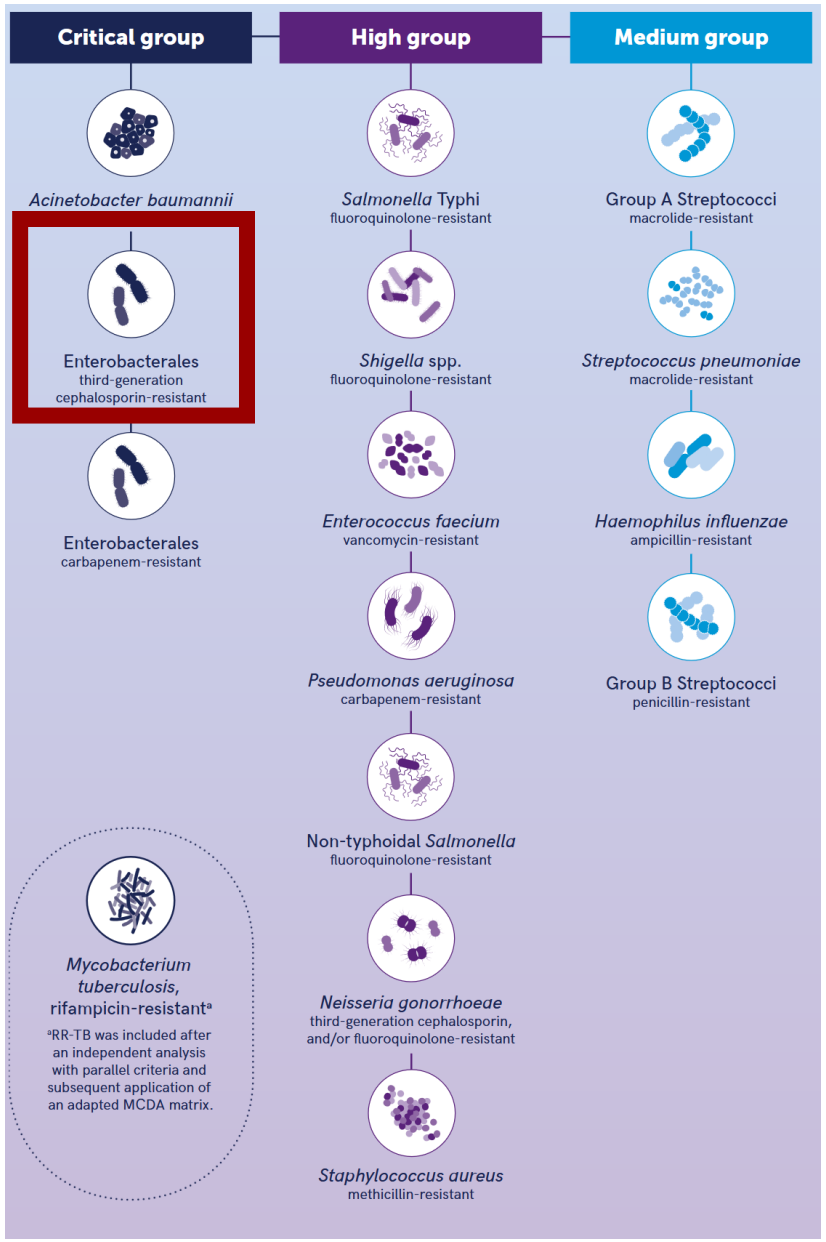
Mikkel Anbo

The effect of environment on antimicrobial resistance

Third-Generation Cephalosporin Resistance in *Escherichia coli* is a critical priority

- Priority pathogens (WHO, EARS-Net)
- CTX-M-15 and CMY-2
- Beta-lactams (penicillins & cephalosporins, etc) are the most widely used antibiotics globally
- Possibly the worst possible abbreviation when combined with ARGs: 3GCRGs

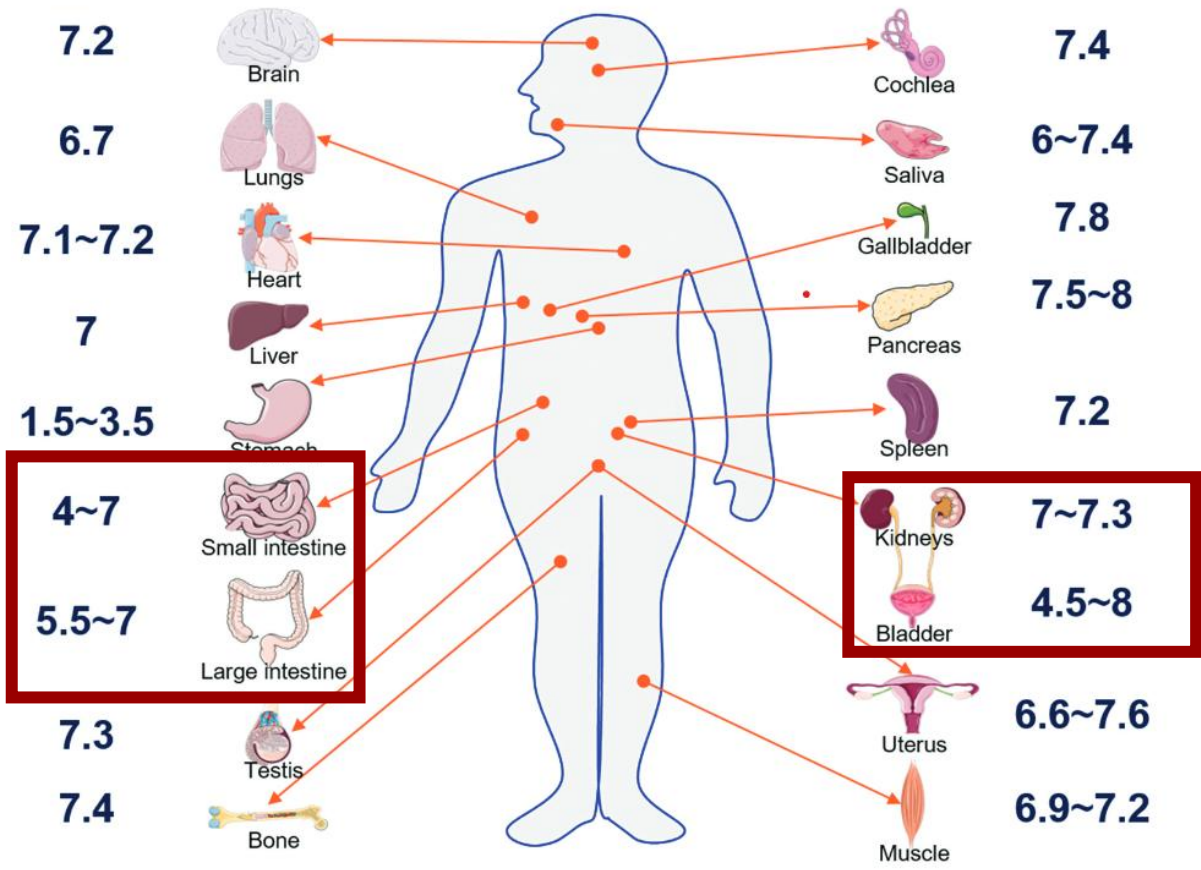
WHO Bacterial Priority Pathogen list, 2024



Is Antimicrobial susceptibility testing accurate enough?

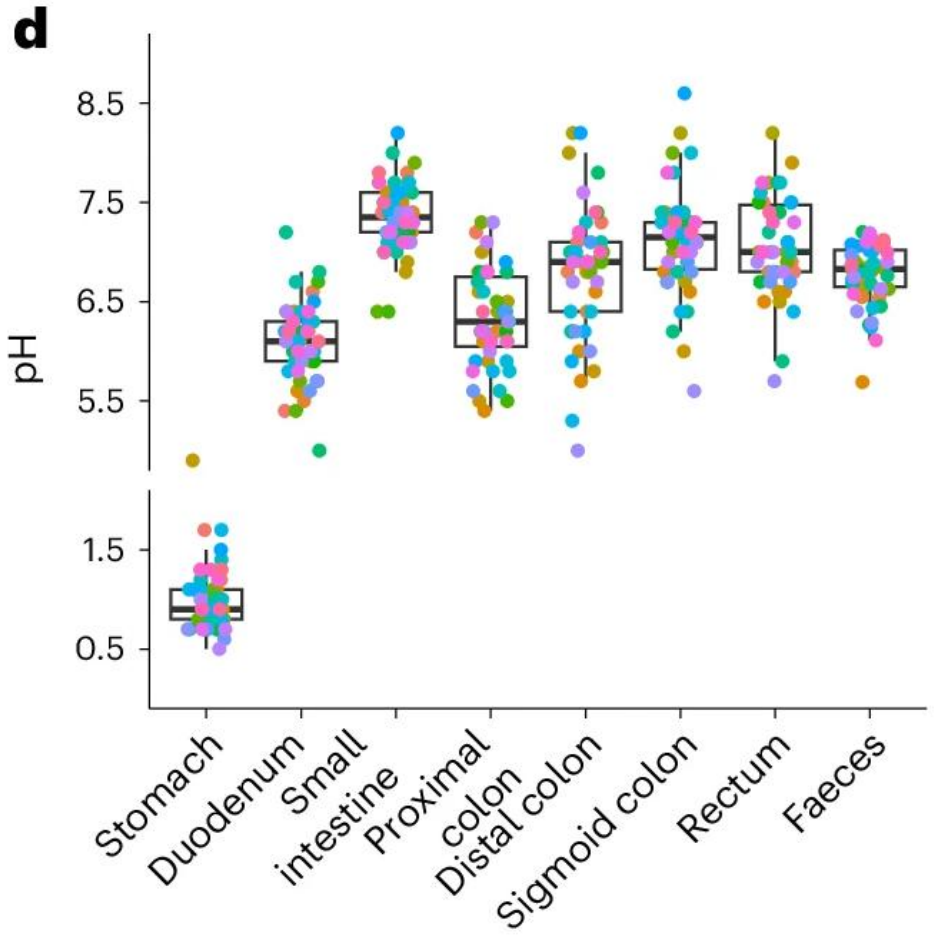
- AMR susceptibility testing done at very standardized condition (pH, temperature, media).
- Numerous environmental factors are overlooked, including pH variation, host-pathogen interactions etc.
- *E. coli* UTI's are associated with acidic urine, while other organisms may alkalize urine with urease
- pH affects the efficacy of several antimicrobial agents

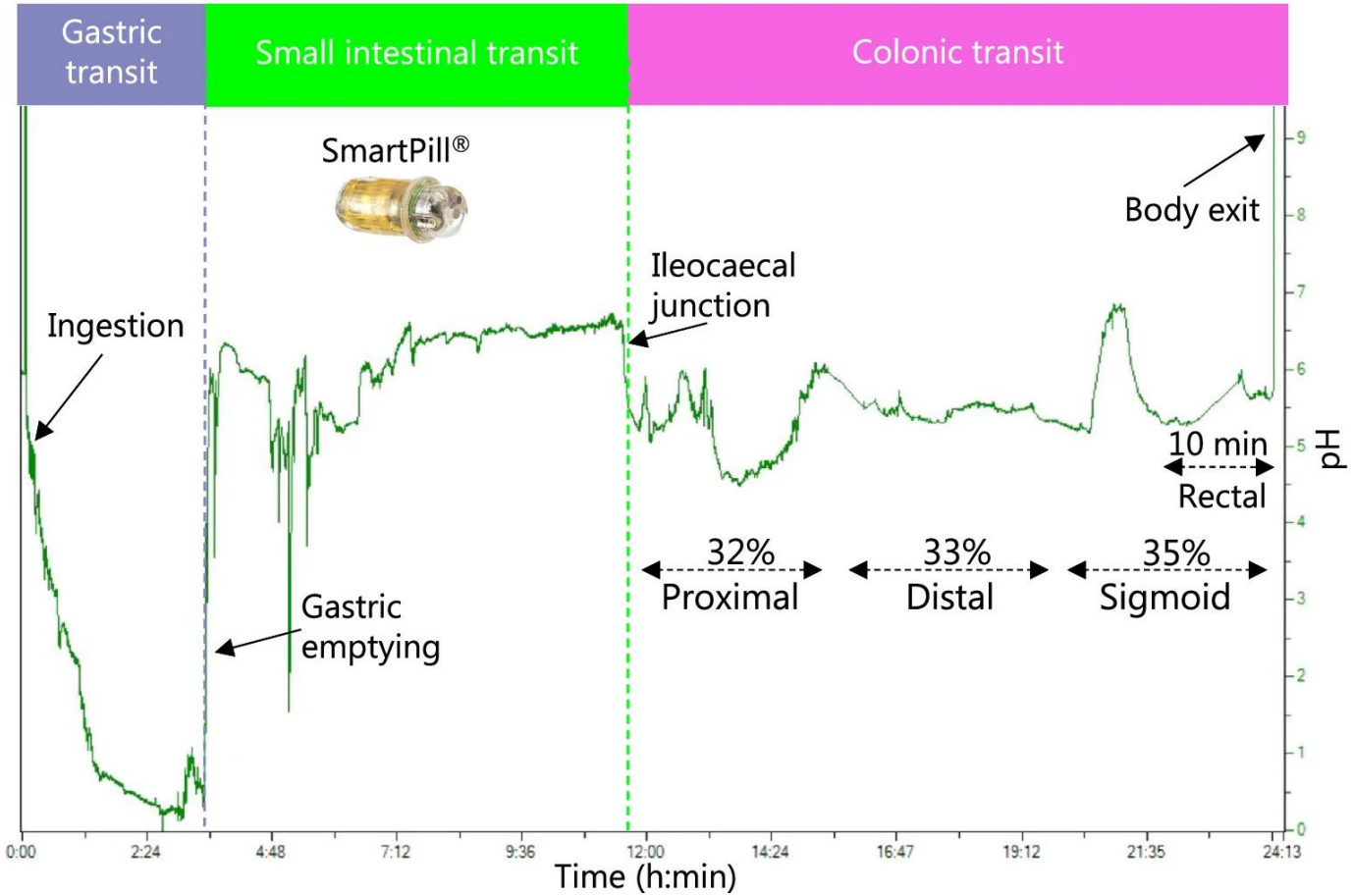
Physiological pH values in the body



Gaohua L, Miao X, Dou L. 2021. Crosstalk of physiological pH and chemical pKa under the umbrella of physiologically based pharmacokinetic modeling of drug absorption, distribution, metabolism, excretion, and toxicity. Expert Opinion on Drug Metabolism & Toxicology.

Large pH variation in the gut





Figures 2d and Extended Data Fig 3.

Procházková, N., Laursen, M.F., La Barbera, G. *et al.* Gut physiology and environment explain variations in human gut microbiome composition and metabolism. *Nat Microbiol* **9**, 3210–3225 (2024). https://doi.org/10.1038/s41564-024-01856-x

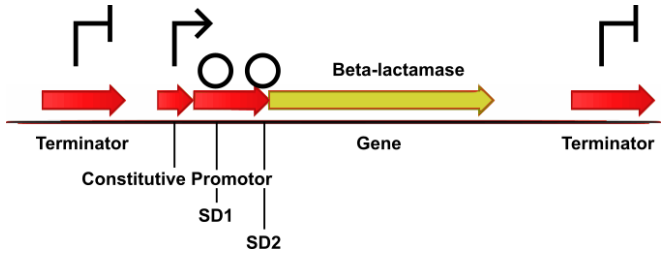
Research questions

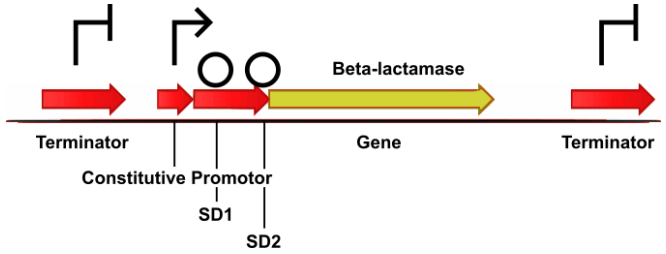
- Is the activity of beta-lactamases impacted by changes in pH? **– Can we treat resistant infections if we modify their environment?**

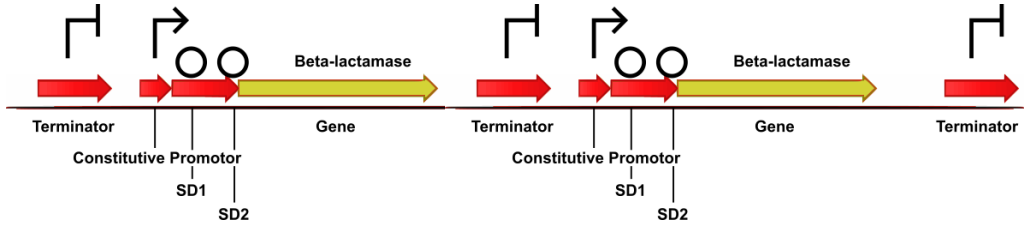
- Does the environment play a role in the success of specific genes and combination of genes? **–Help us better understand the evolution and dissemination of AMR**

ß-lactamase expressing strains

- CTX-M: **CTX**, BLUE
- CMY: **CMY**, RED
- K12, **wildtype**, GREEN
- CTX-M & CMY: **CMYCTX**, PURPLE

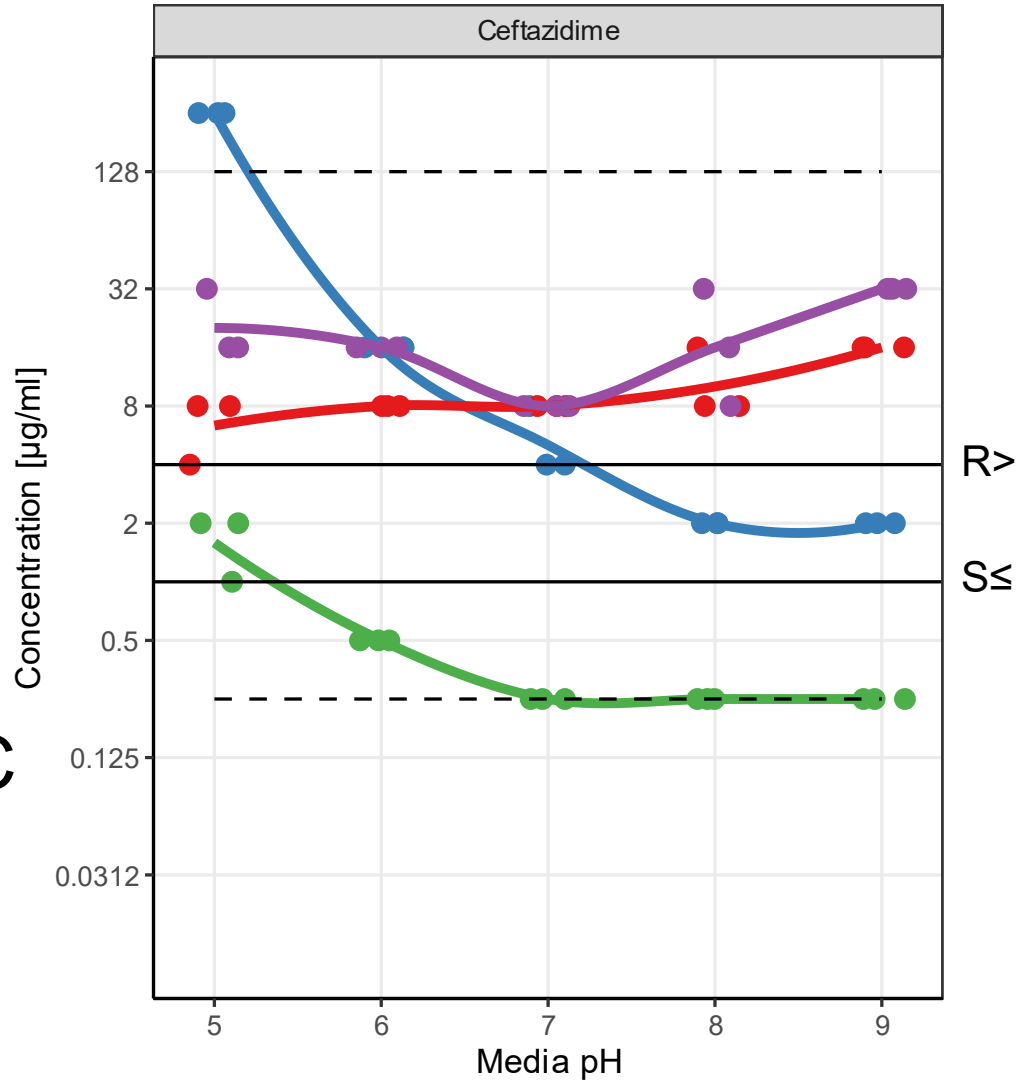






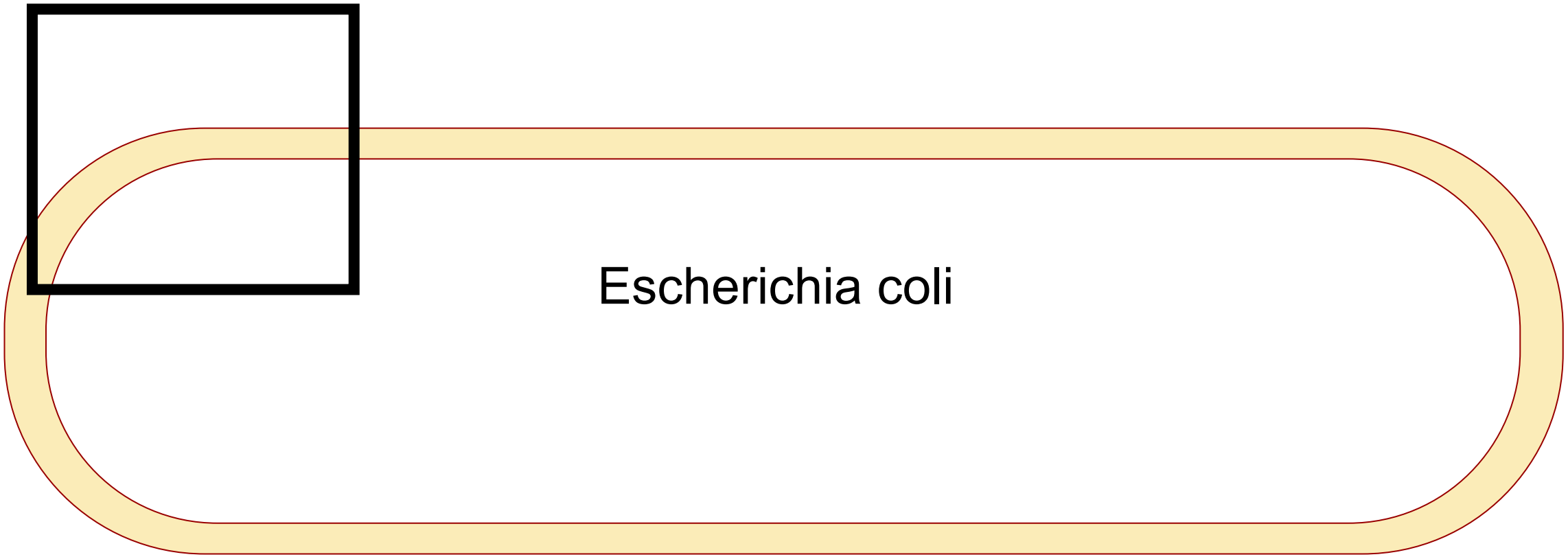
CTX-M-15 and CMY-2 have contrasting MIC peaks to ceftazidime

- CTX-M:
  high MIC at low pH
- CMY
  high MIC at high pH
- CMYCTX MIC at or
  Above single gene MIC

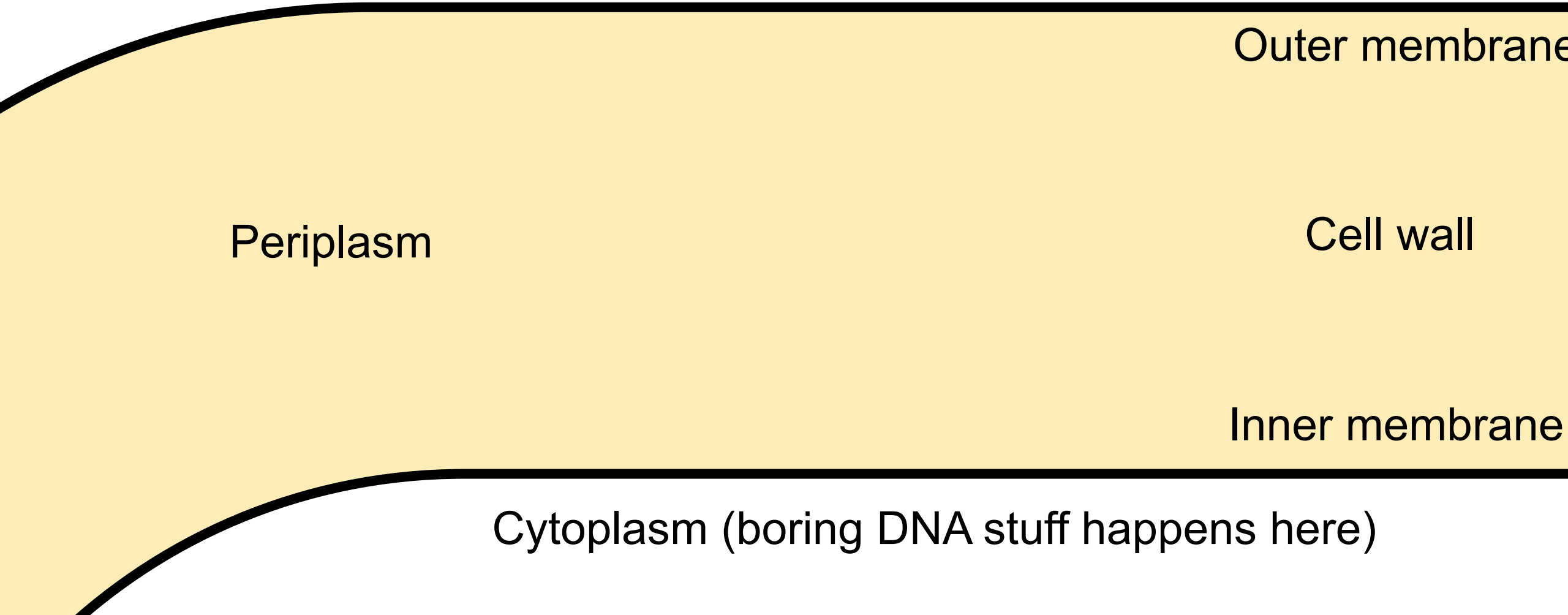


Anbo et al, Contrasting pH optima of β-lactamases CTX-M and CMY influence *Escherichia coli* fitness and resistance ecology, *in press* 2025

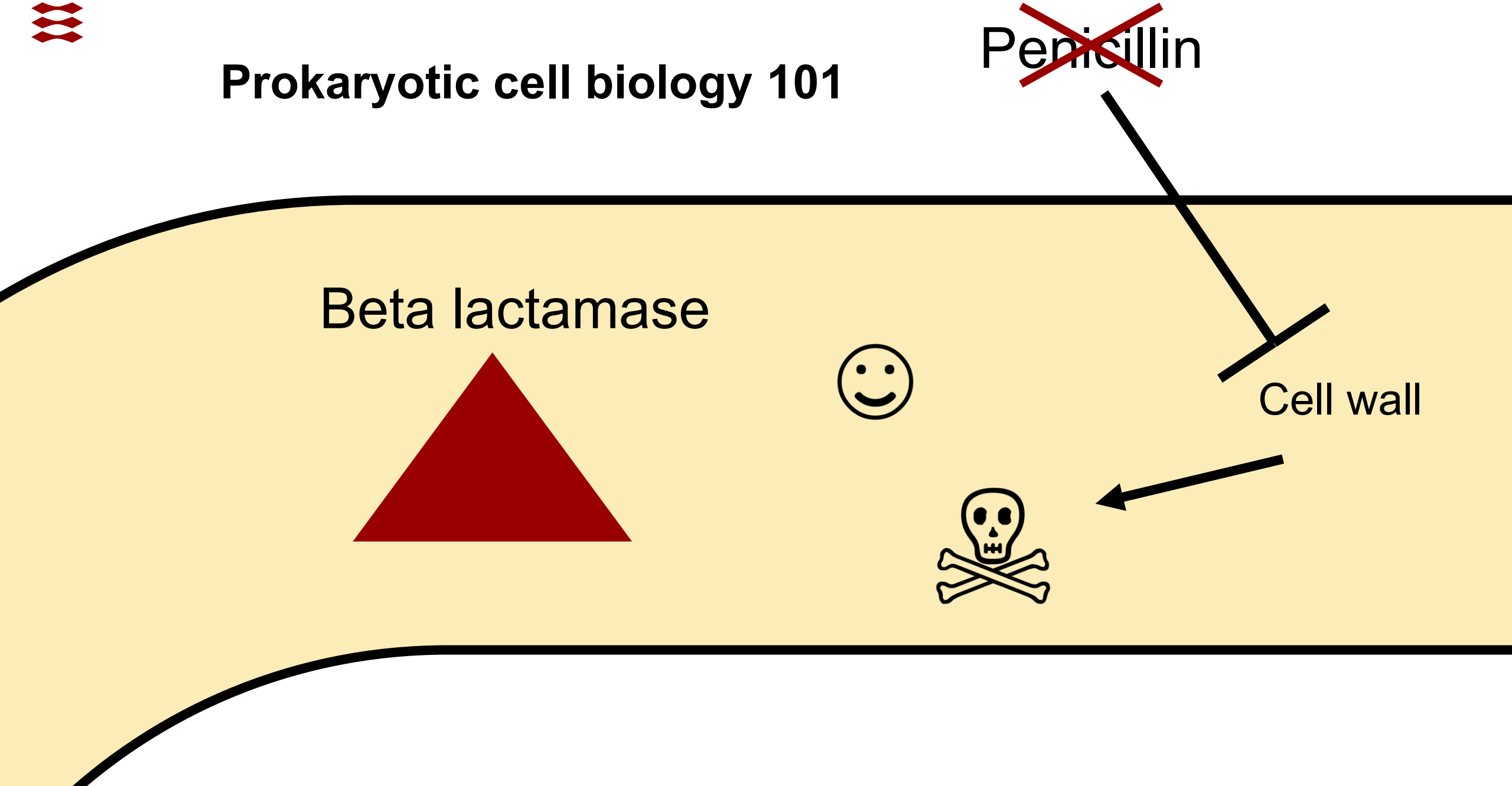
What happens in bacteria during beta lactam treatment?

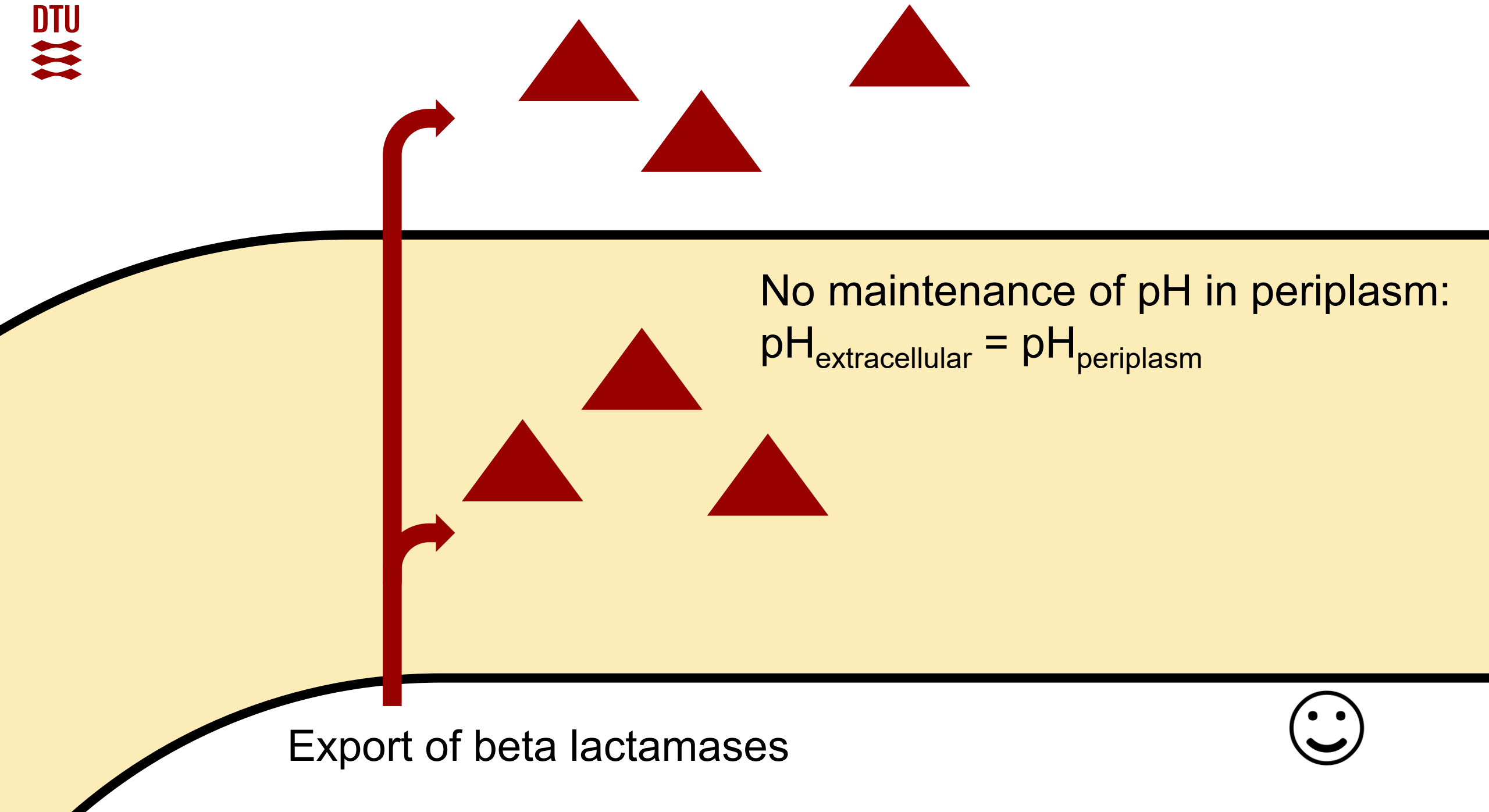


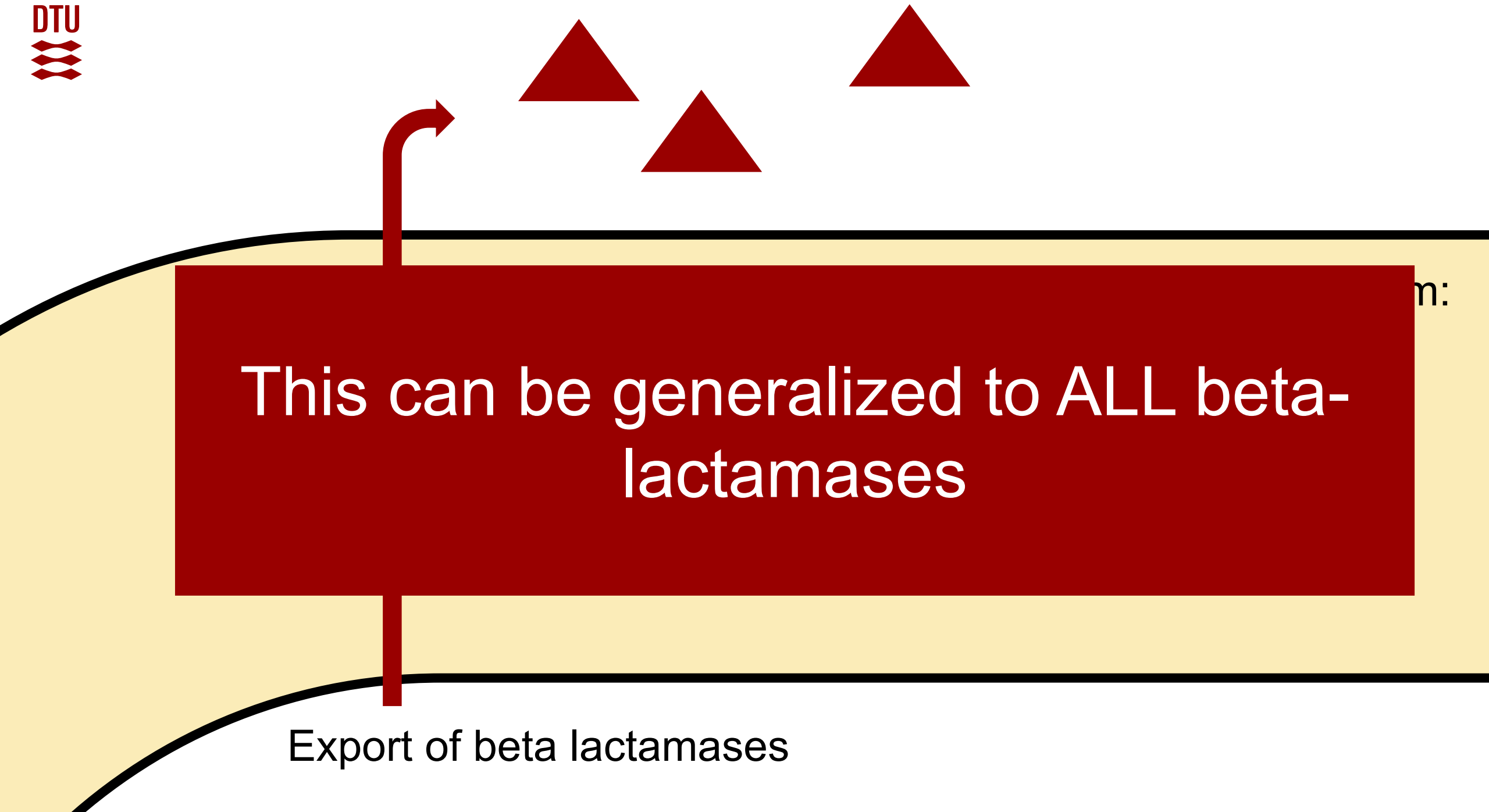
Prokaryotic cell biology 101



Prokaryotic cell biology 101




No maintenance of pH in periplasm:
$pH_{extracellular} = pH_{periplasm}$
Export of beta lactamases


m:
This can be generalized to ALL beta-lactamases
Export of beta lactamases

How does this relate to their fitness?

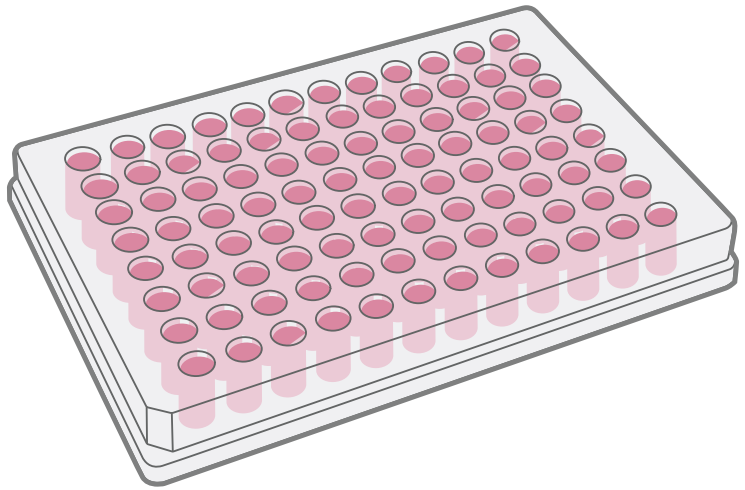
Determining the fitness of beta-lactamases using kinetic growth curves

Culture bacteria at pH's 5-9

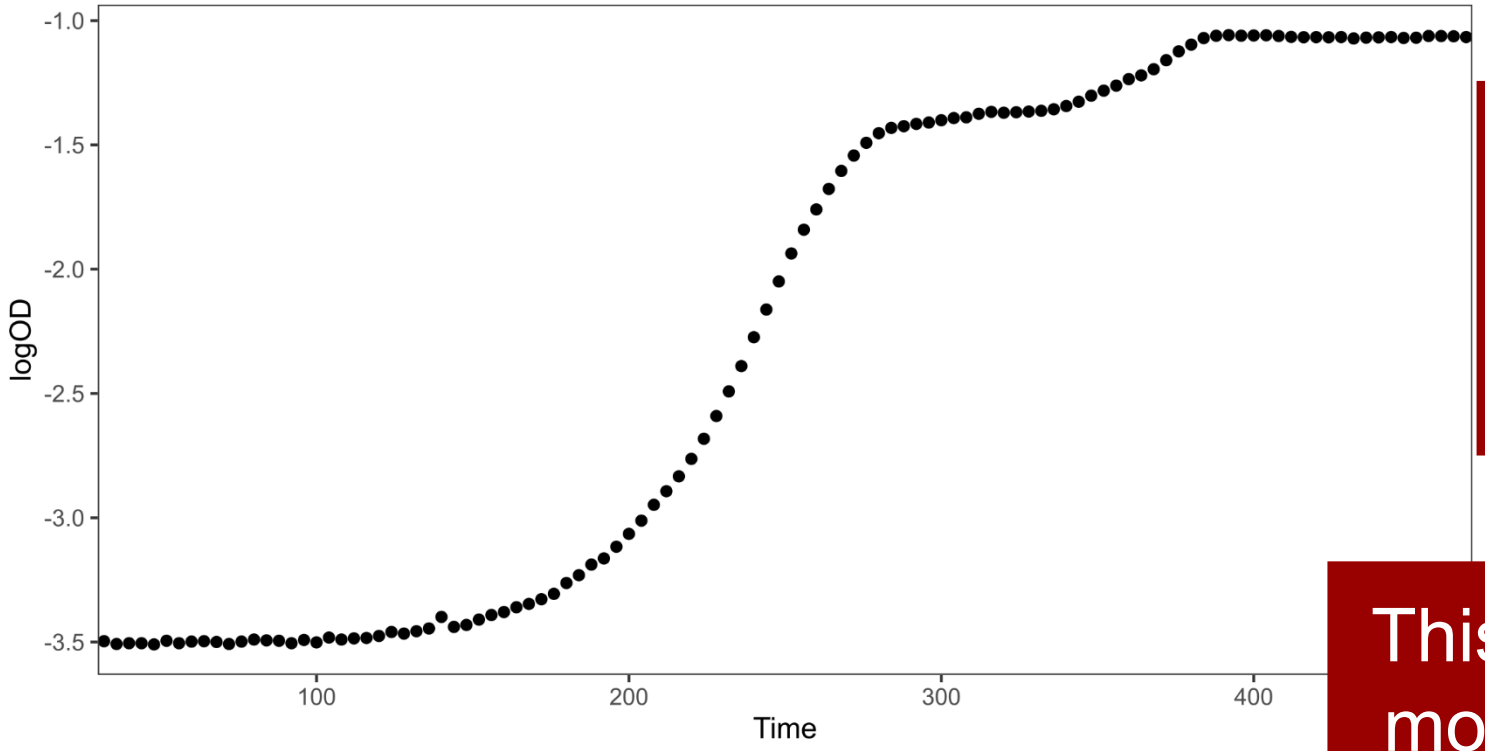
Measure Bacterial density (OD) every 4 minutes

Culture bacteria at pH's 5-9

And at 10 different concentrations of TAZ



Kinetics of strains

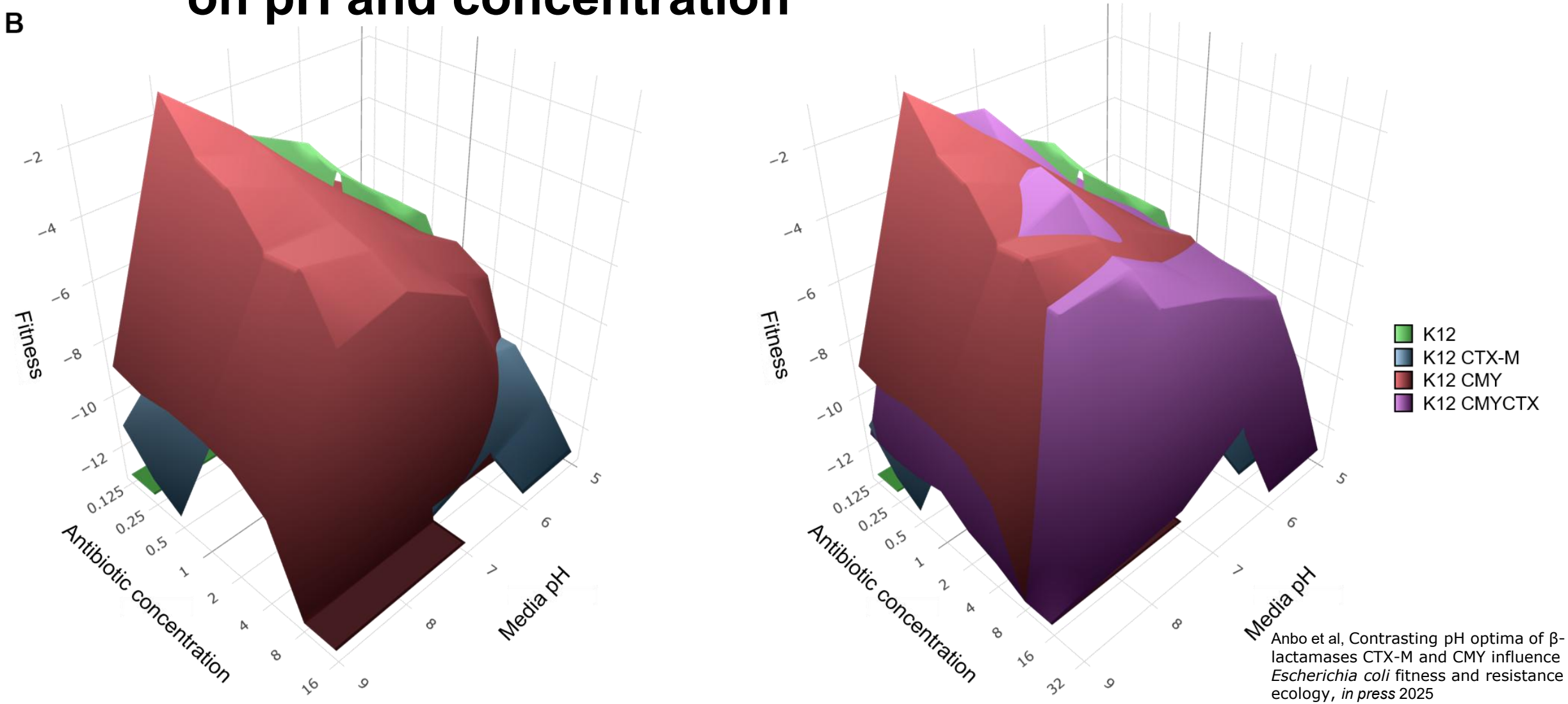


- 4 Strains
- 5 pH's 5-9
- 10 Ceftazidime conc. 0-64µg/ml

For each Strain/pH/Concentration combination we have a growth rate and a lag time

This enables us to formulate a model to predict the fitness of each beta lactamase in a given environment

Fitness landscape of CTX-M and CMY: dependency on pH and concentration



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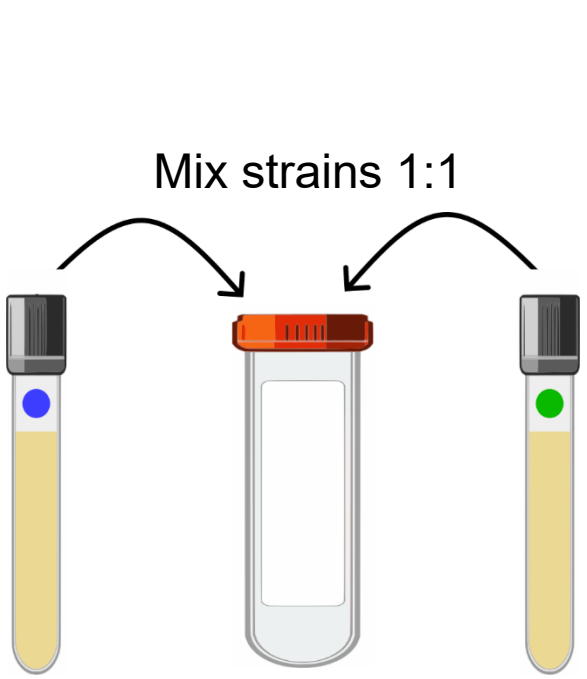
Co-culture: CTX-M vs CMY



Mix strains 1:1

4x

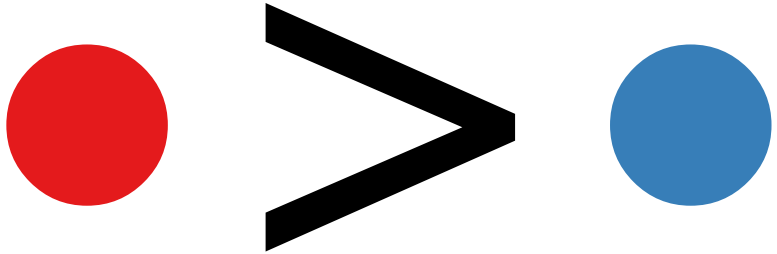
Grow 24 h at pH 5 or 8
+4-16 µg/ml Ceftazidime

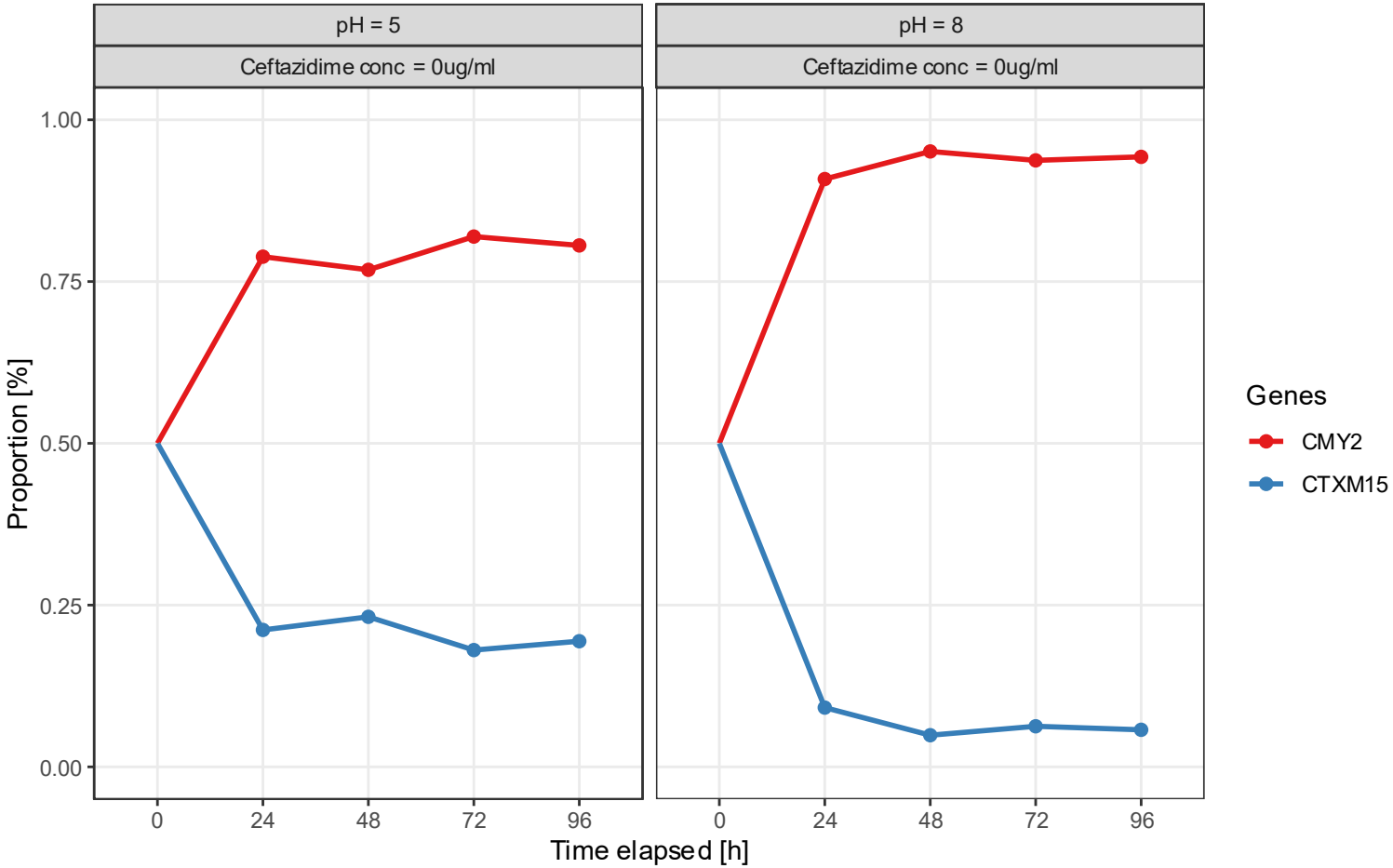
Passage to fresh
media every x hour

Sample & DNA
purify

Sequencing
&
KMA-ing

Co-culture without ceftazidime





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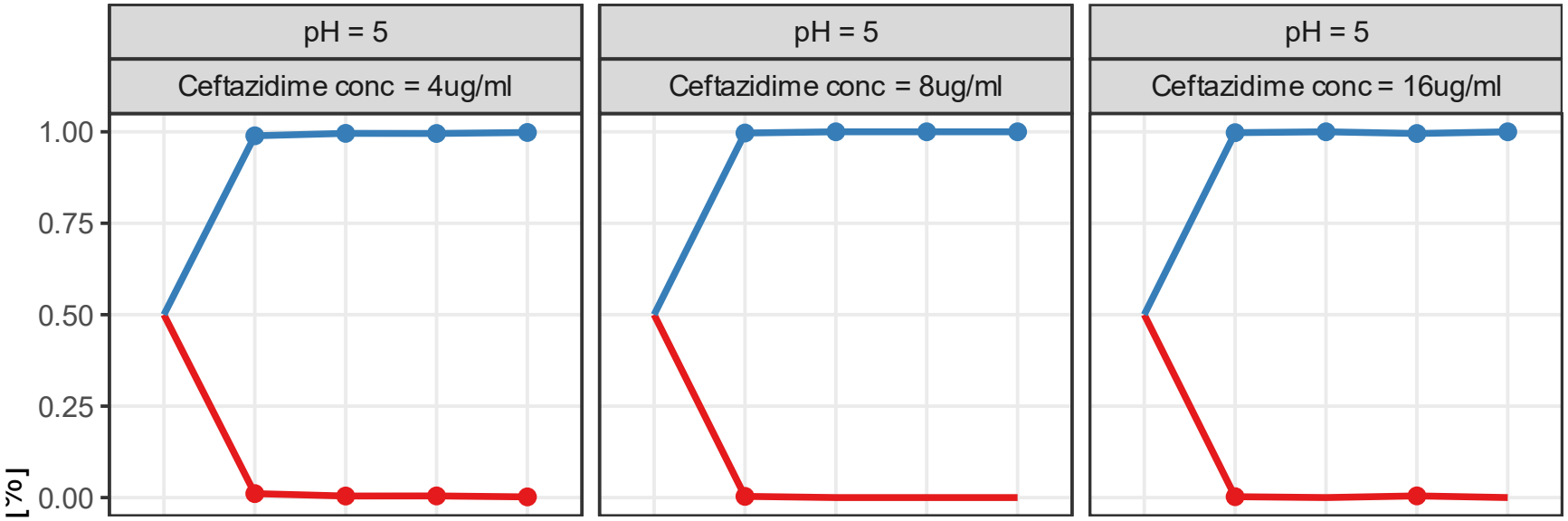
Co-culture with ceftazidime

CTX-M
successful at low pH
>= 4 µg/ml TAZ



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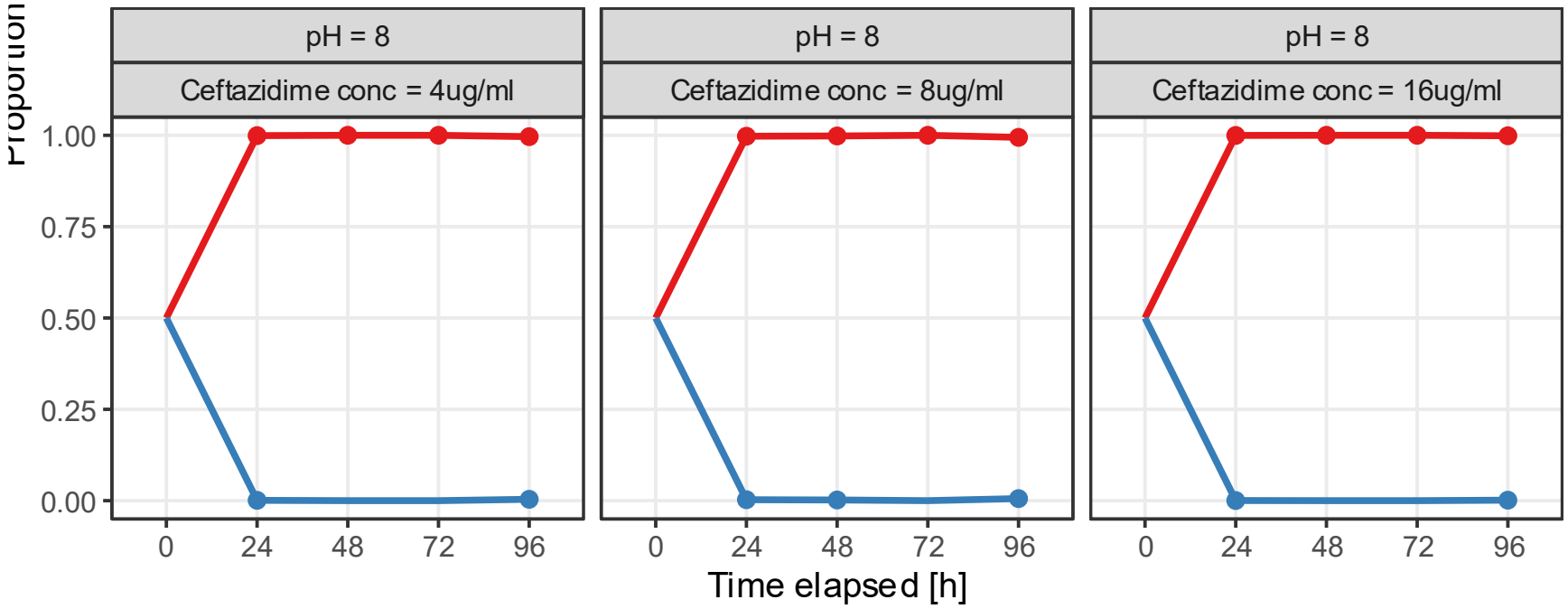
Co-culture with ceftazidime

CMY
successful at high pH
>= 4 µg/ml TAZ



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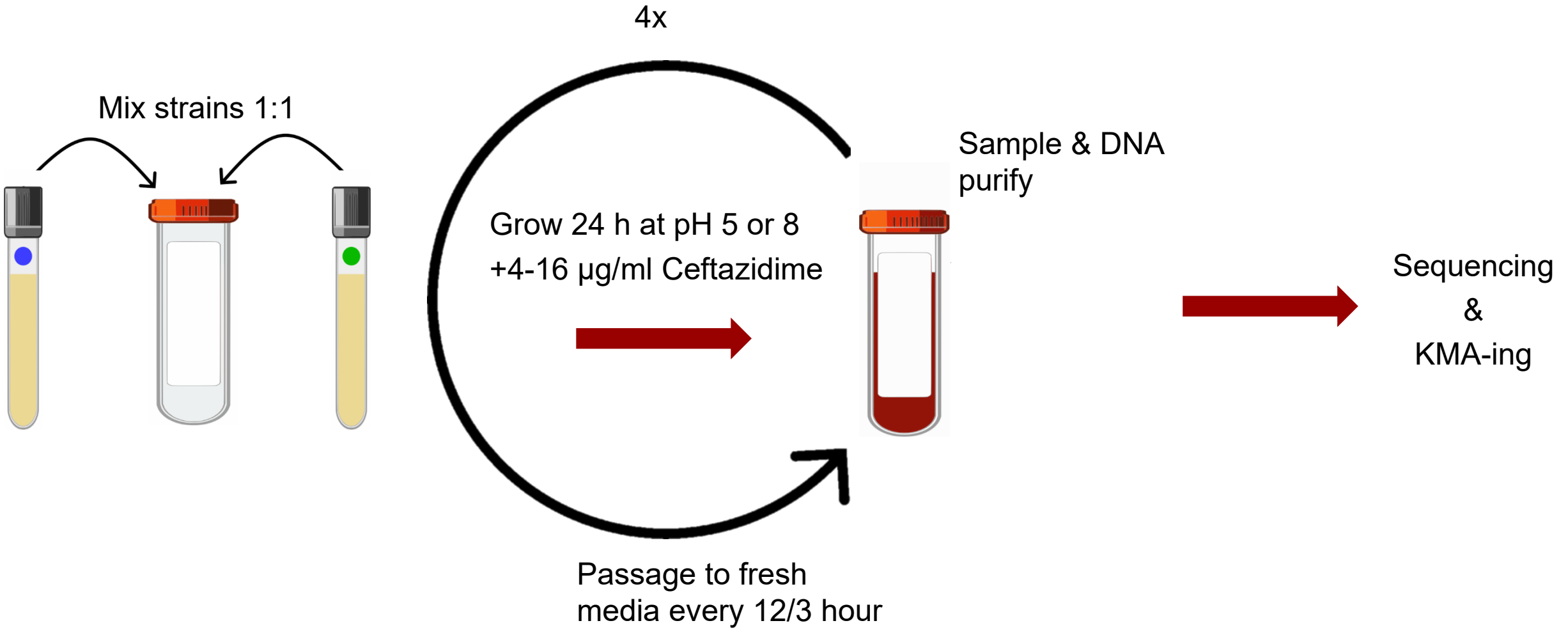
Co-culture: CTX-M vs CMY



Long story short (and a lot of co-cultures)

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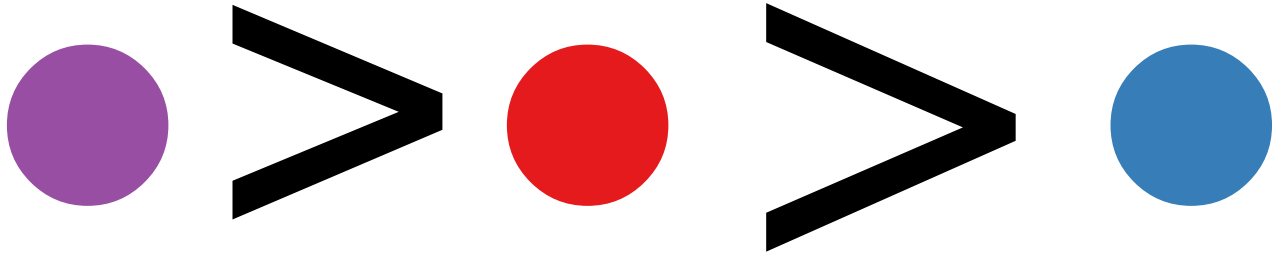
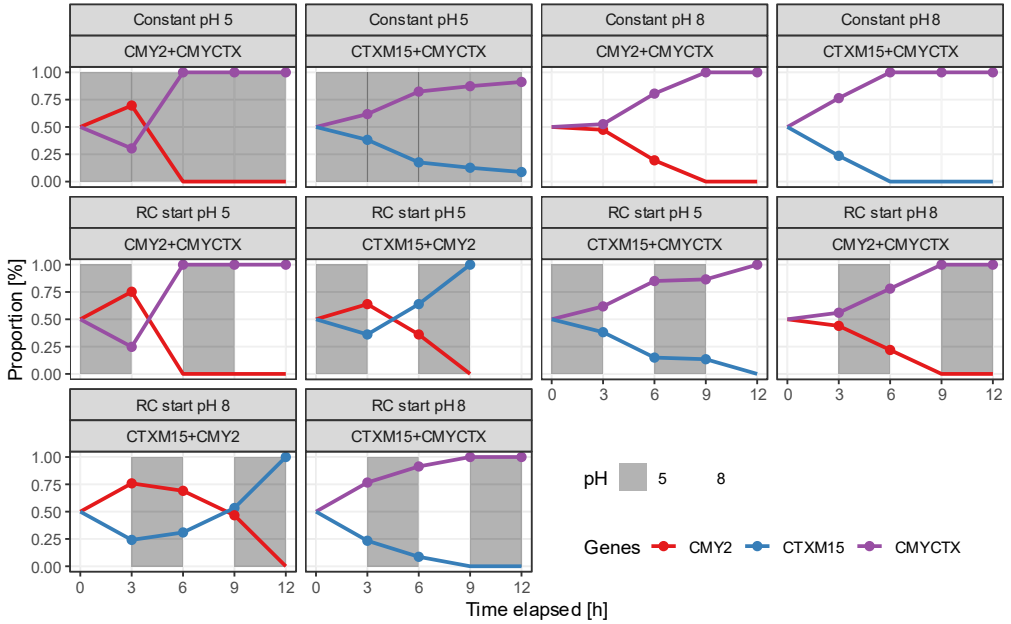
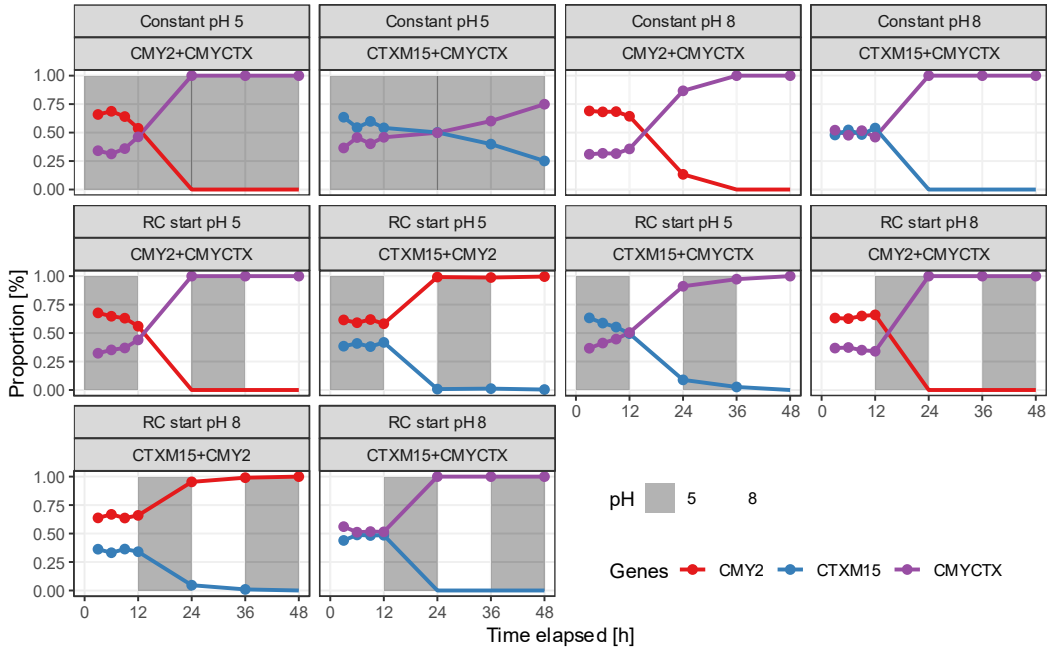
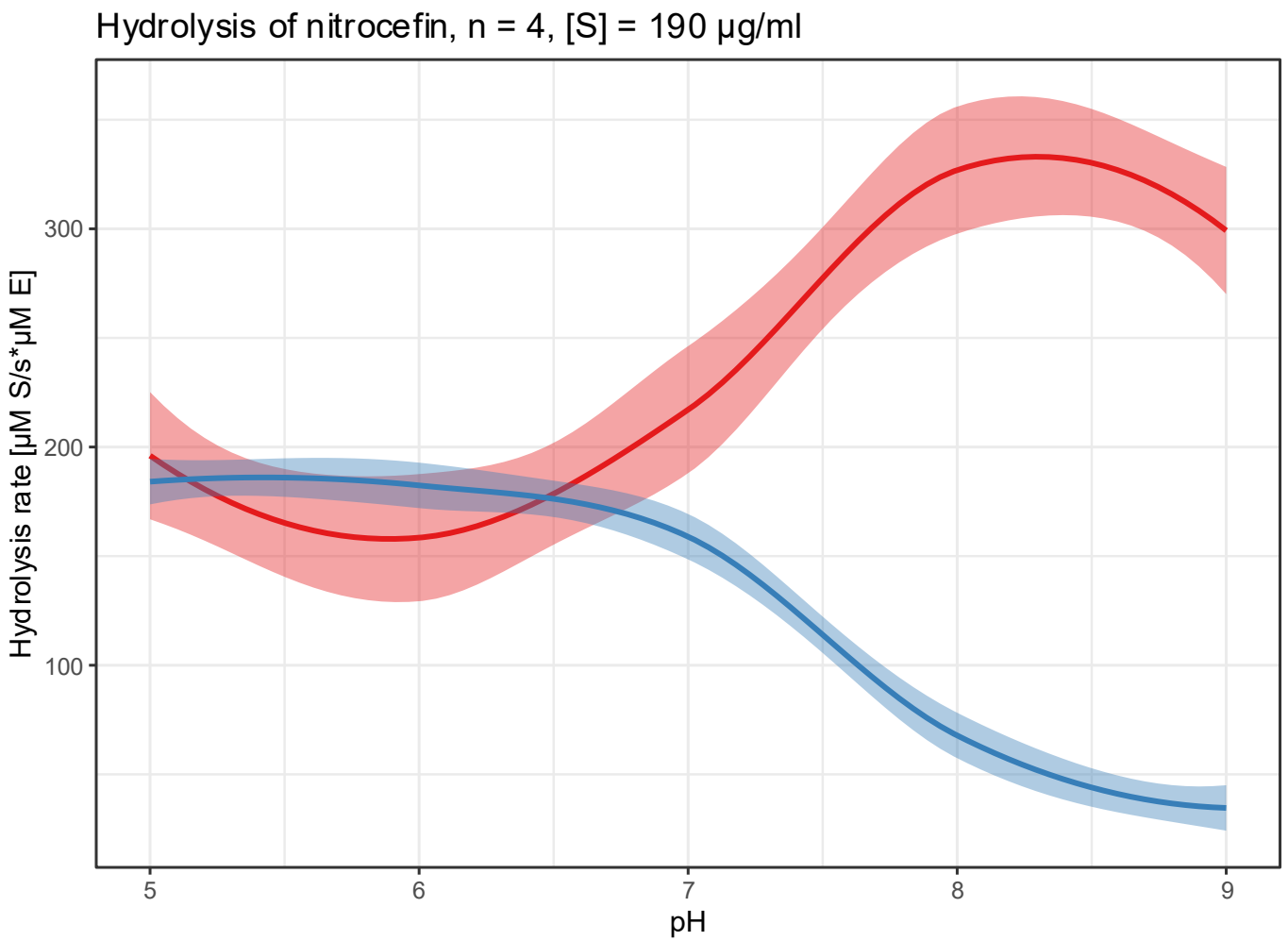


But why?





Nitrocefin hydrolysis rates



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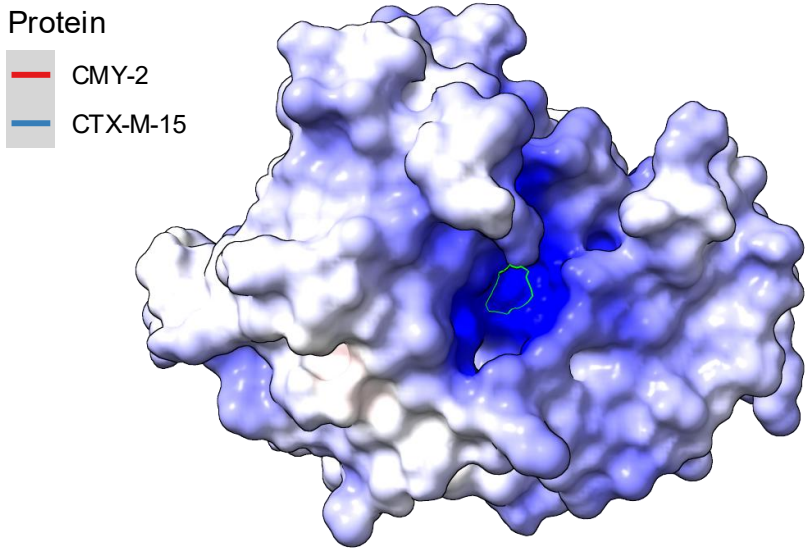
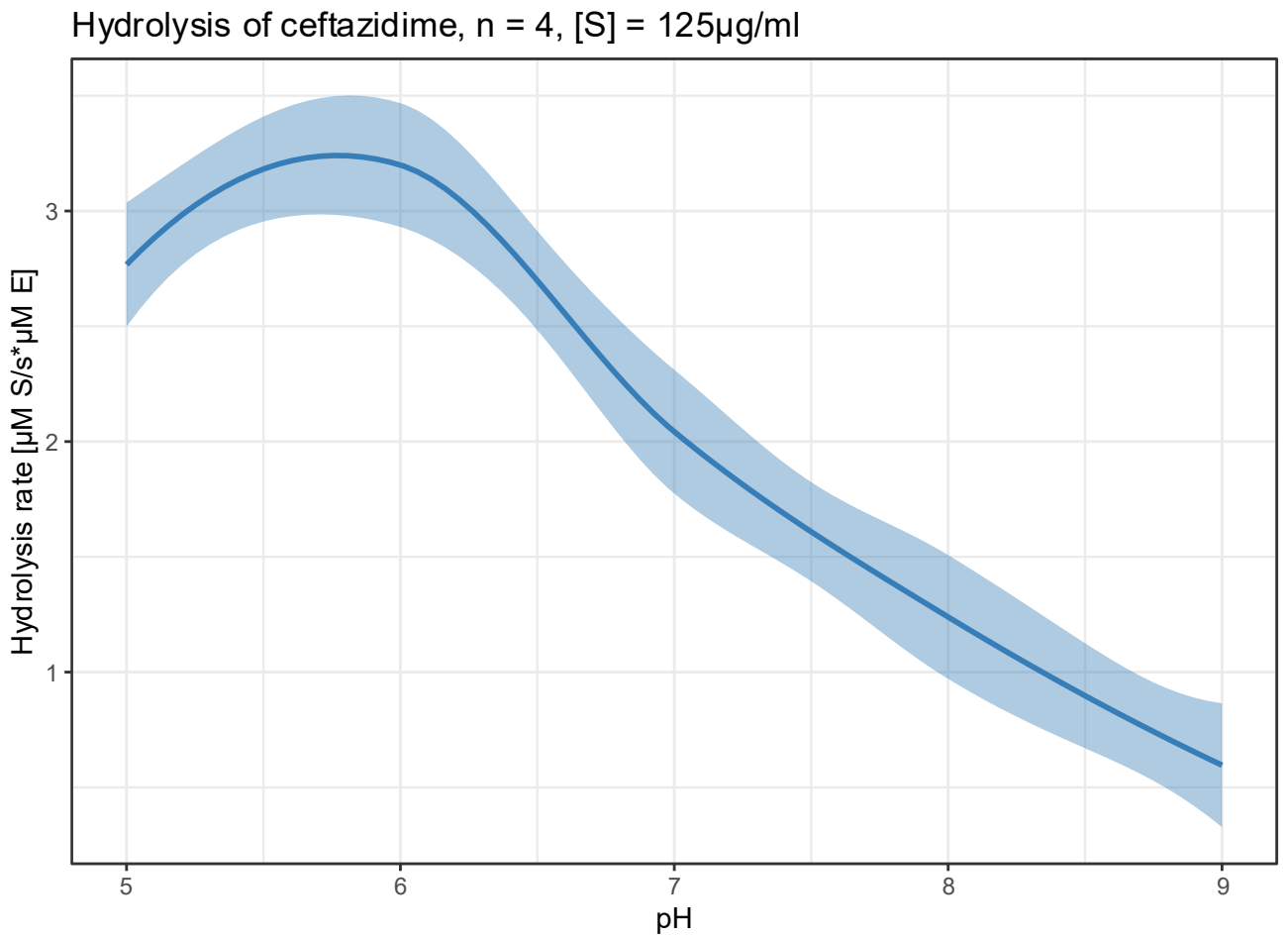


But it’s not the right substrate…

Ceftazidime hydrolysis rate



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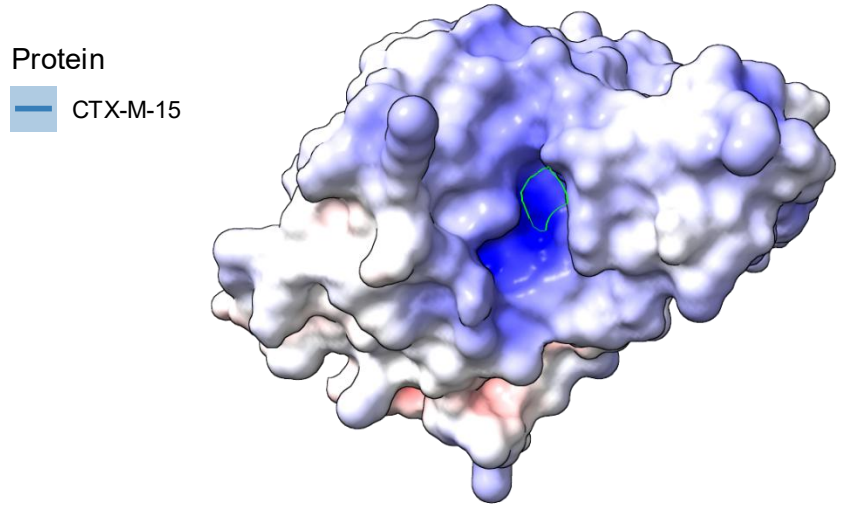
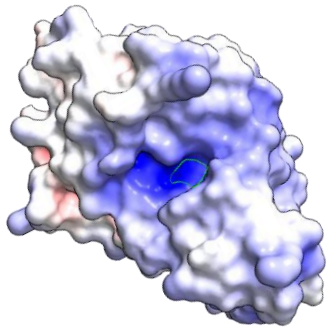
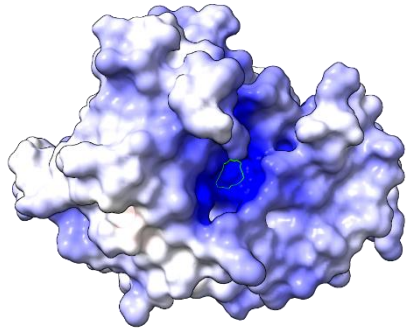


We cant measure *in vitro* hydrolysis of ceftazidime with CMY-2 ☹

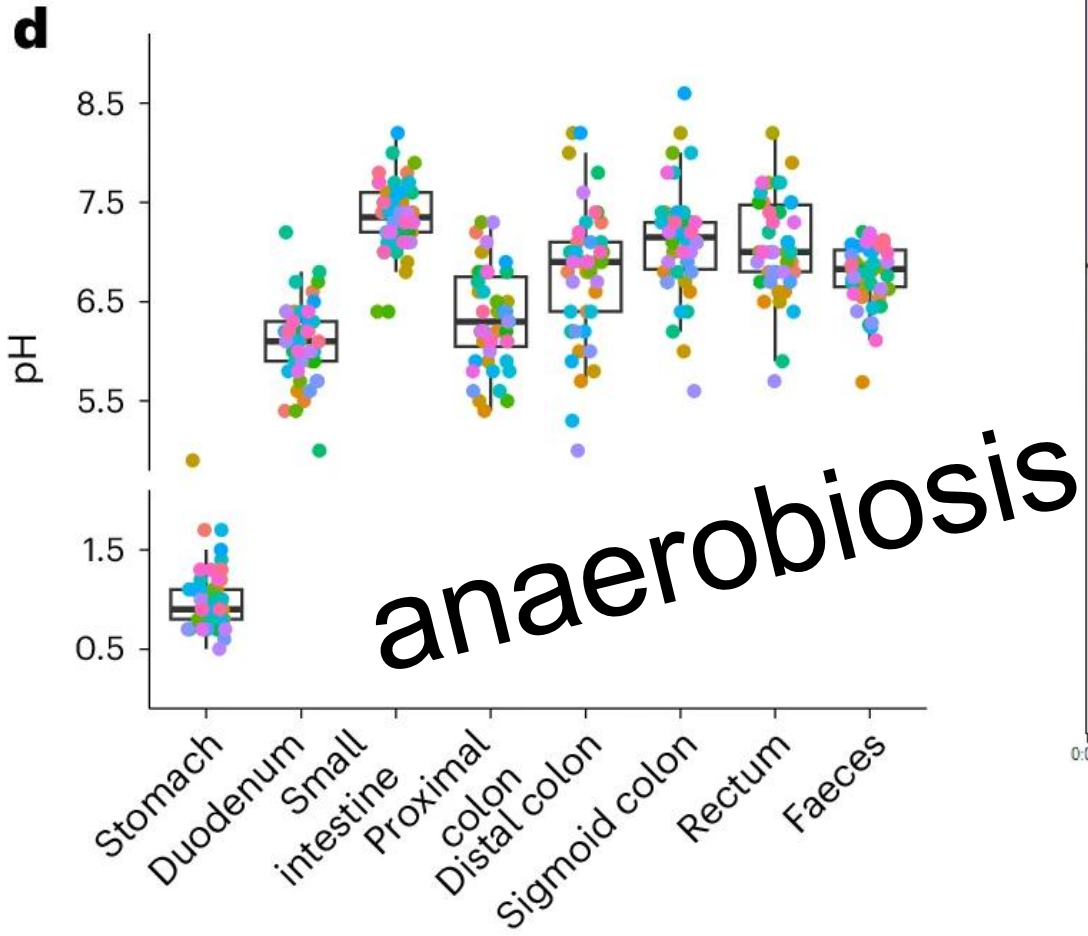
pH influences E coli fitness and resistance ecology how?

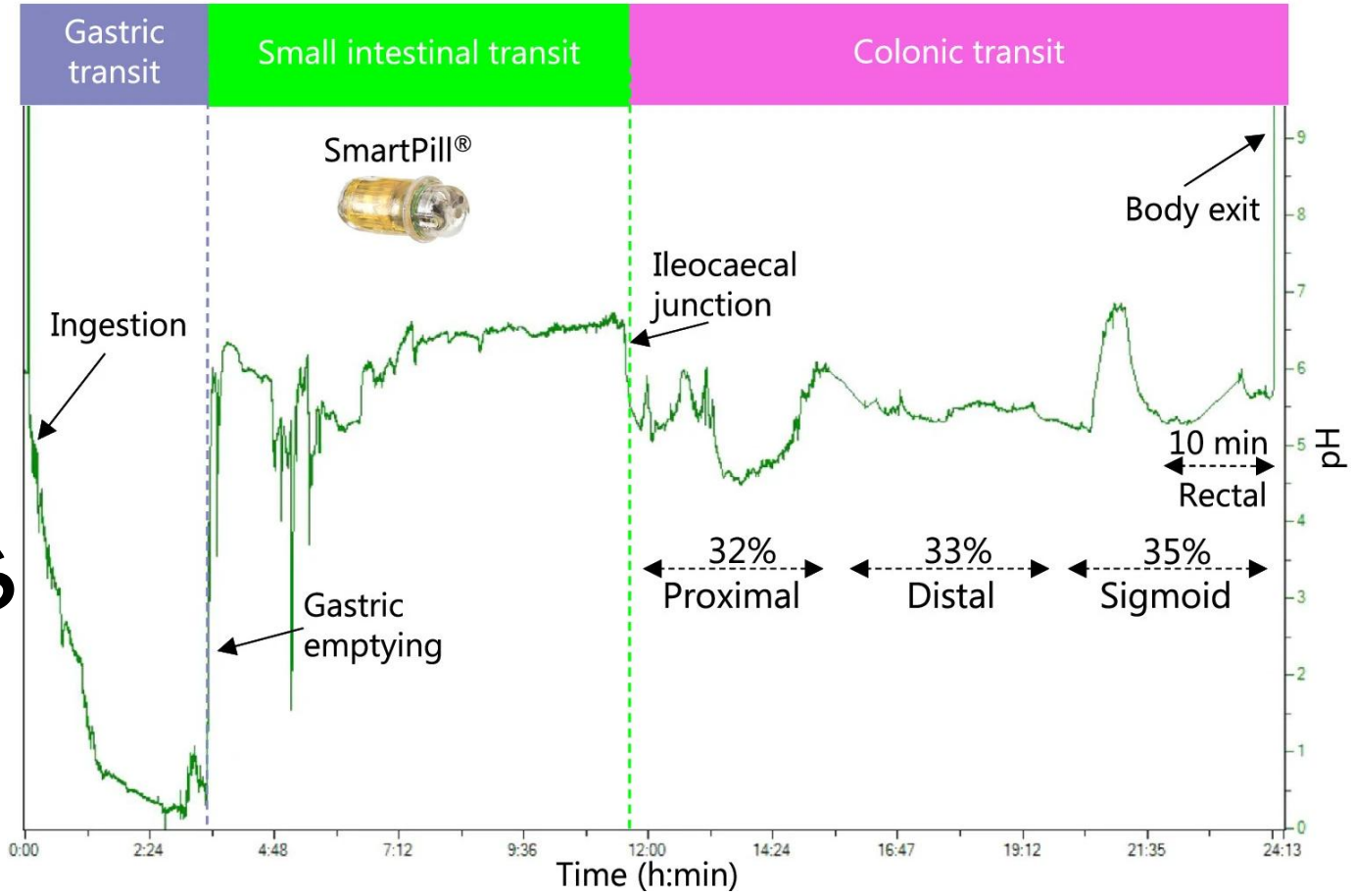
- CTX-M-15 may impose an inherently higher fitness cost than CMY-2
- Enzyme kinetics and stability may explain the differentiating effect of pH on ecology
- Changing environments could explain why isolates amass redundant beta-lactamases (between different patients, environments, niches etc)





Other (defining) environmental effects in the gut?





Figures 2d and Extended Data Fig 3.

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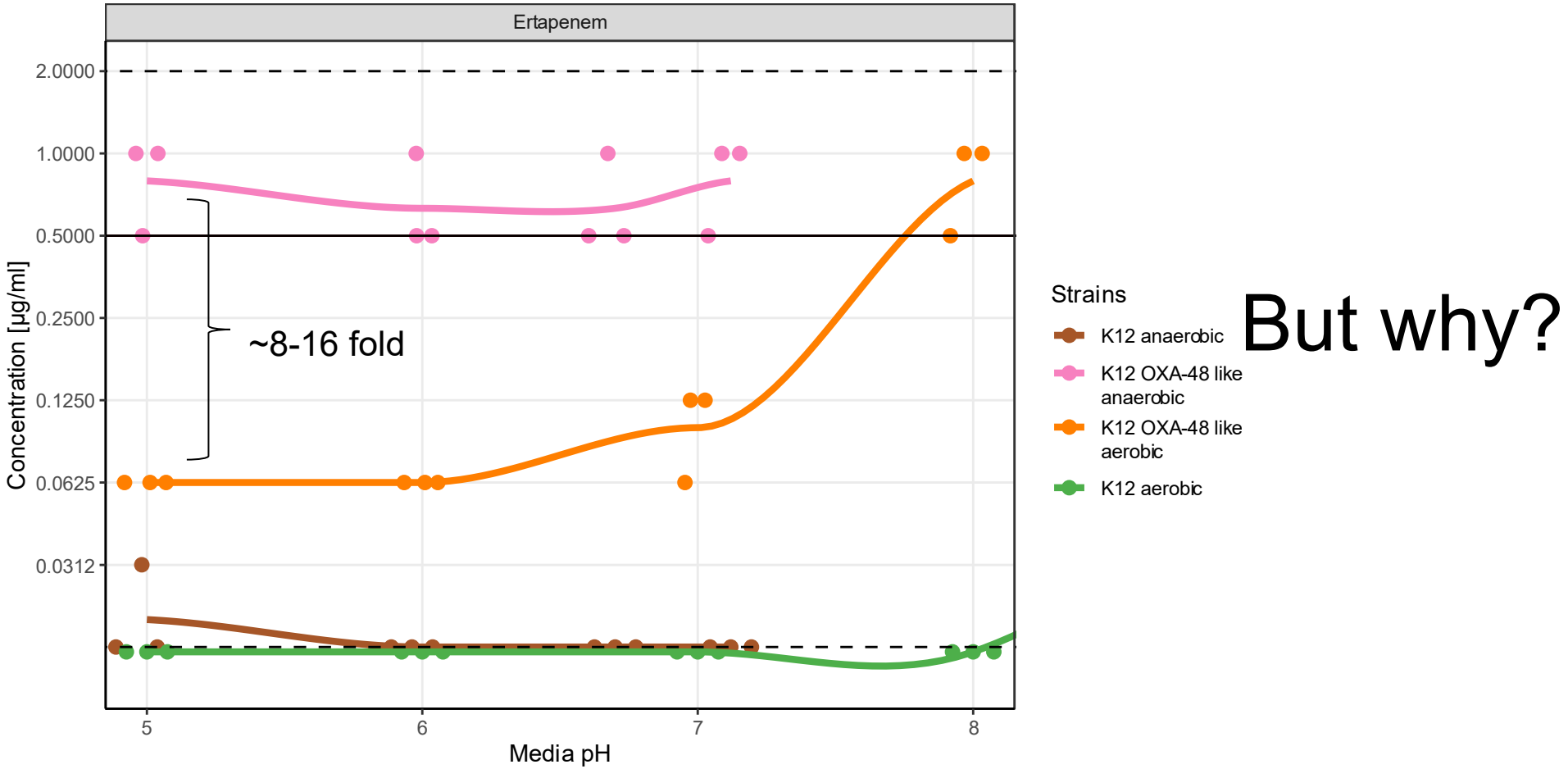
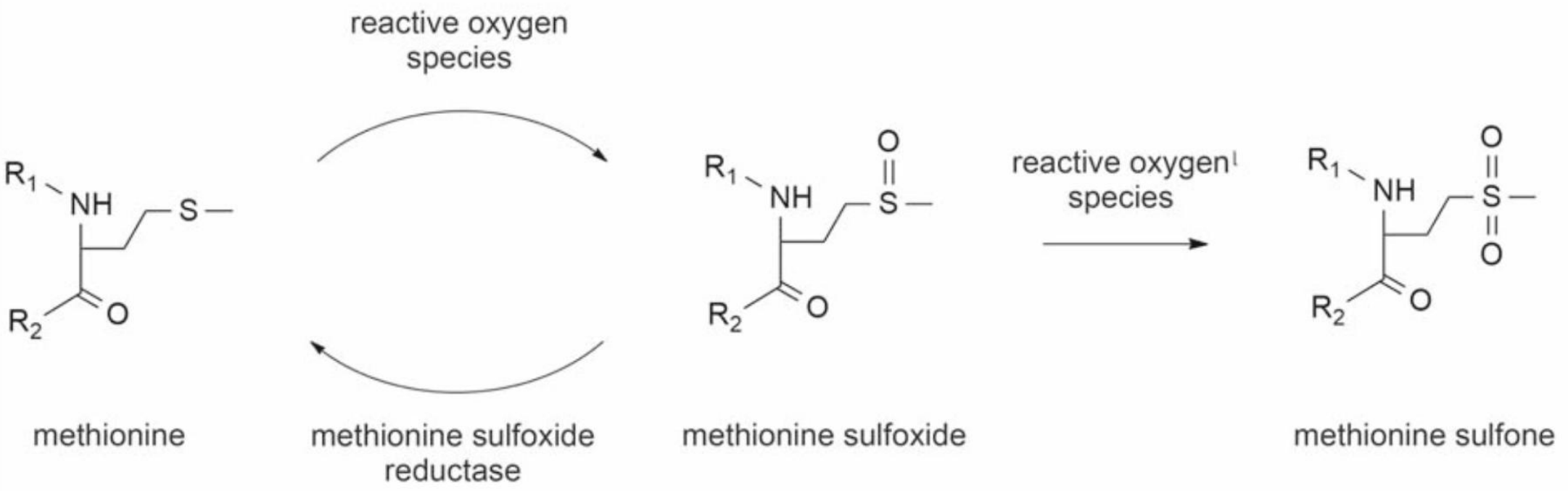
blaOXA-48 like gene confers resistance to ertapenem during anaerobiosis



But why?

What is oxygen if not simply redox potential?



msrA in *E. coli* (not the macrolide resistance one)

Pohanka, M. "Oxidative stress in Alzheimer disease as a target for therapy." *Bratisl. Lek. Listy* 119.9 (2018): 535-543.

5 “redox-active” residues near active site:

Met115

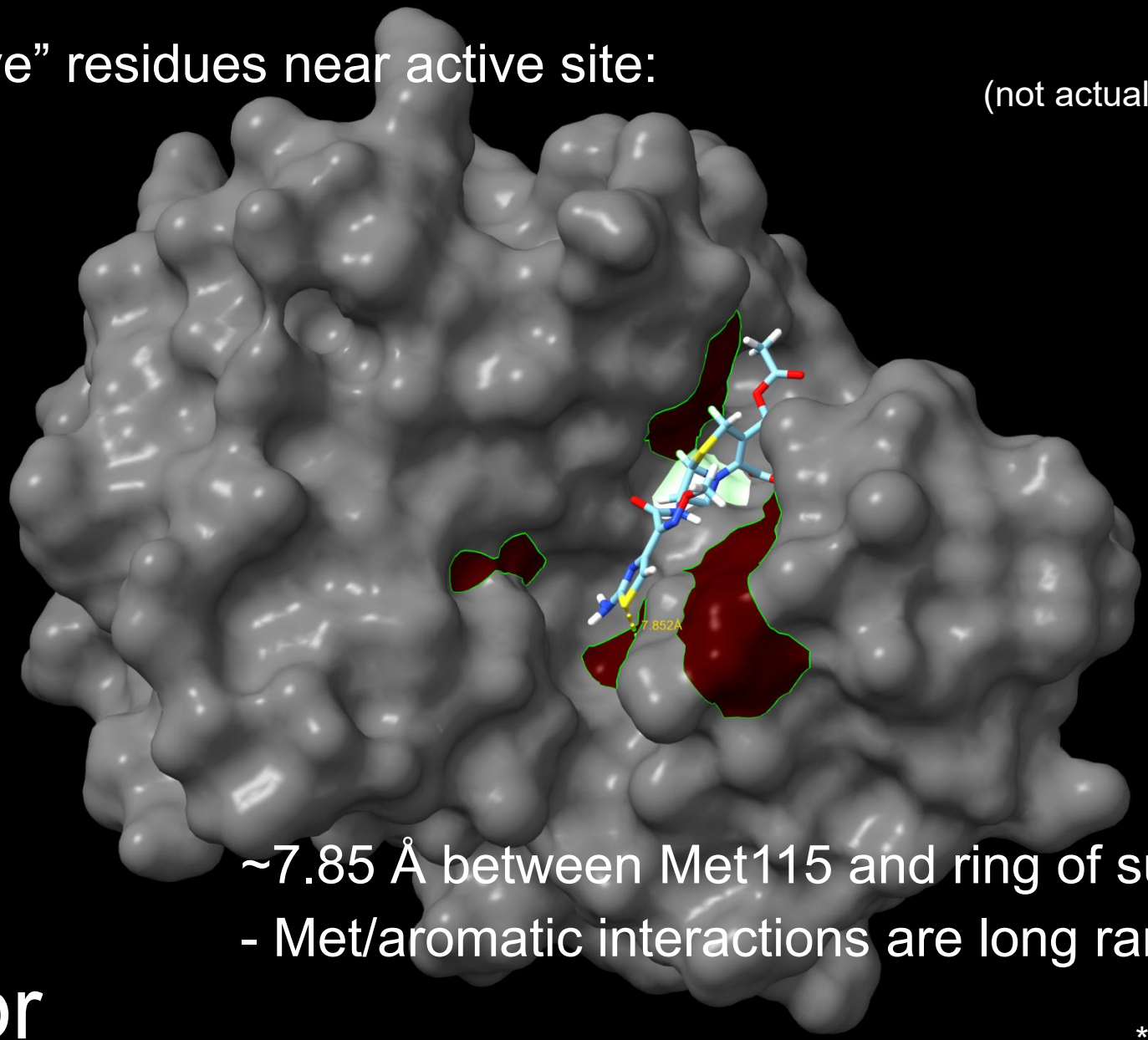
Tyr117

Trp105

Tyr211

Trp222

(not actually molecular docking)



OXA-48

Pdb: 3hbr

~7.85 Å between Met115 and ring of substrate

- Met/aromatic interactions are long range up to 8.5Å*

*Orabi & English 2018

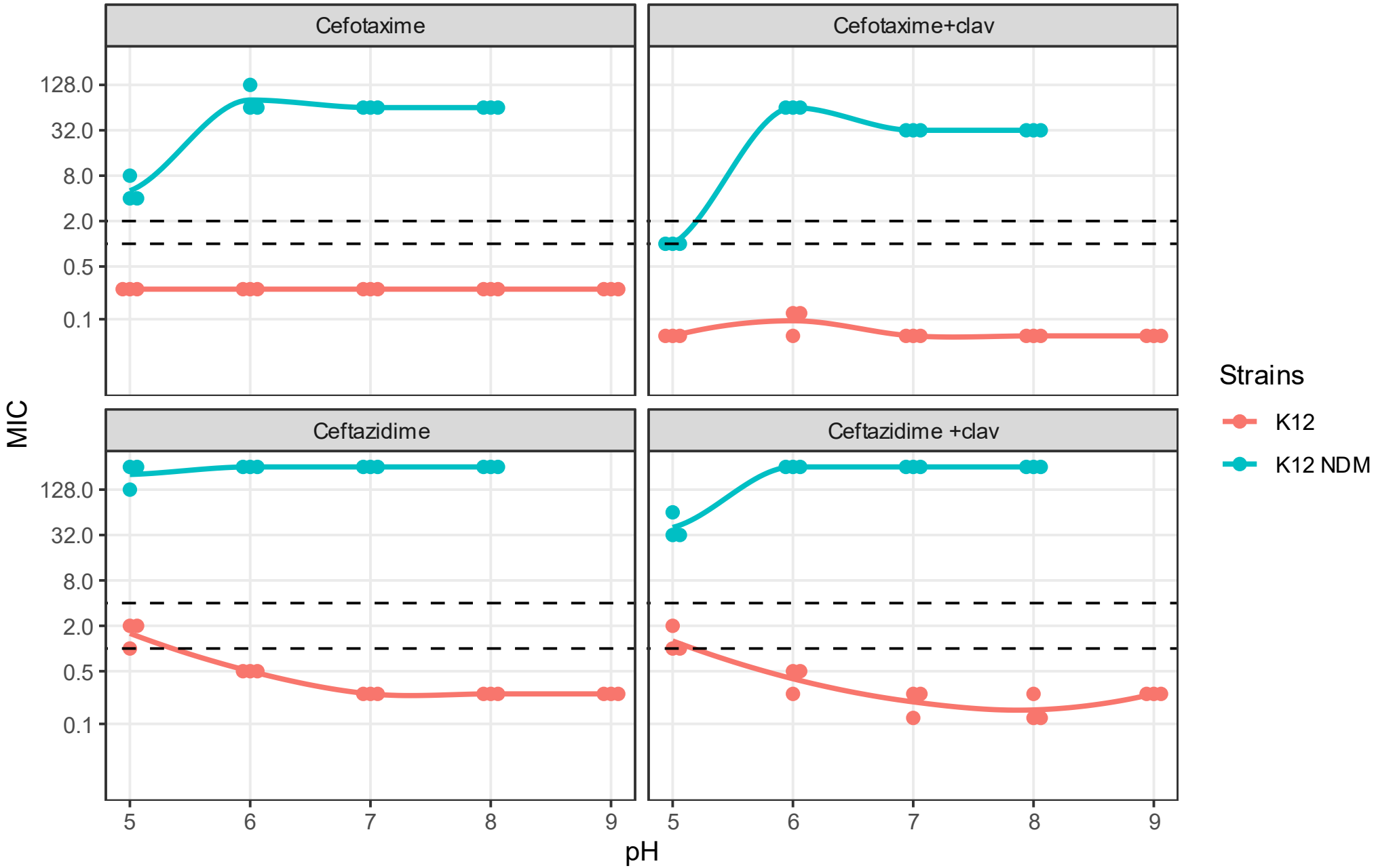
Hypothesis: Met115 in OXA is oxidized, rendering it a “poor” carbapenemase

- M115 is 100% conserved among all OXA-48 like beta lactamases (total 67 variants)
- Proximal to the active site, within reasonable distance (~8Å) to the substrate
- Reduced methionine stabilizes ertapenem binding unlike its oxidized form

Can we test this?

- Enzyme kinetics in the presence/absence of a reducer (e.g. DTT)
- Substitute M115Q to simulate oxidation and test MIC +/- oxygen

Beta lactamase inhibitors seem to work on NDM at low pH



Thanks for listening