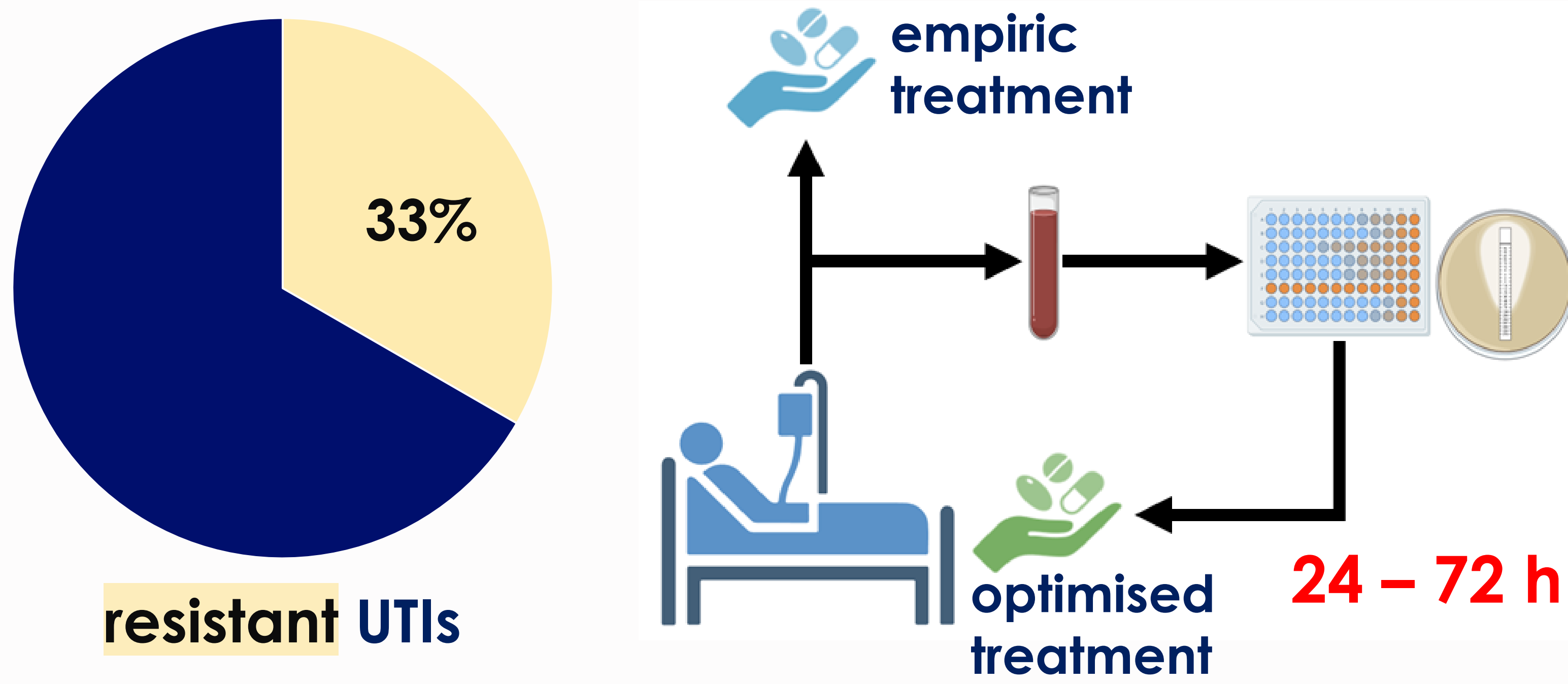


Bacterial electrophysiology for rapid diagnosis of antimicrobial resistance

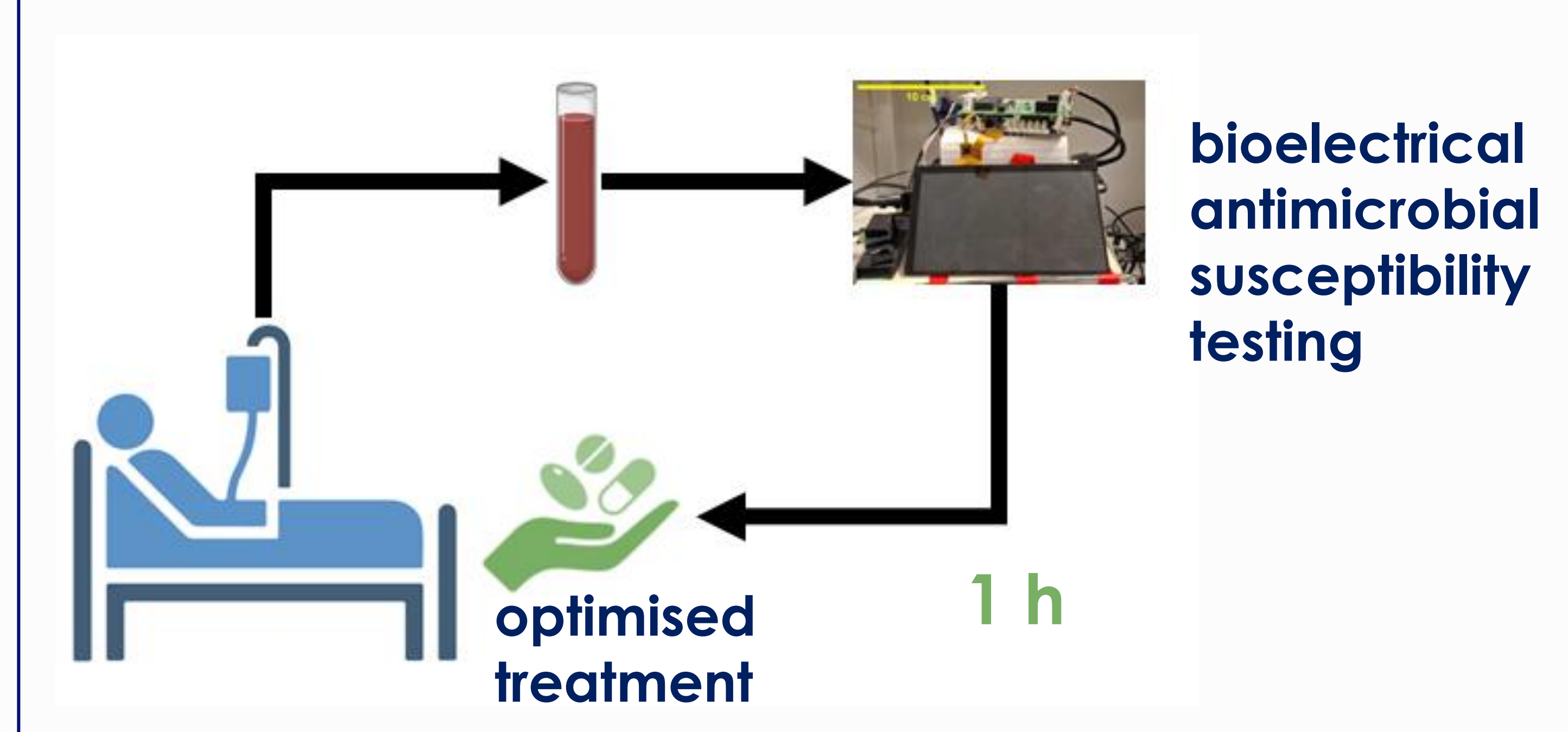
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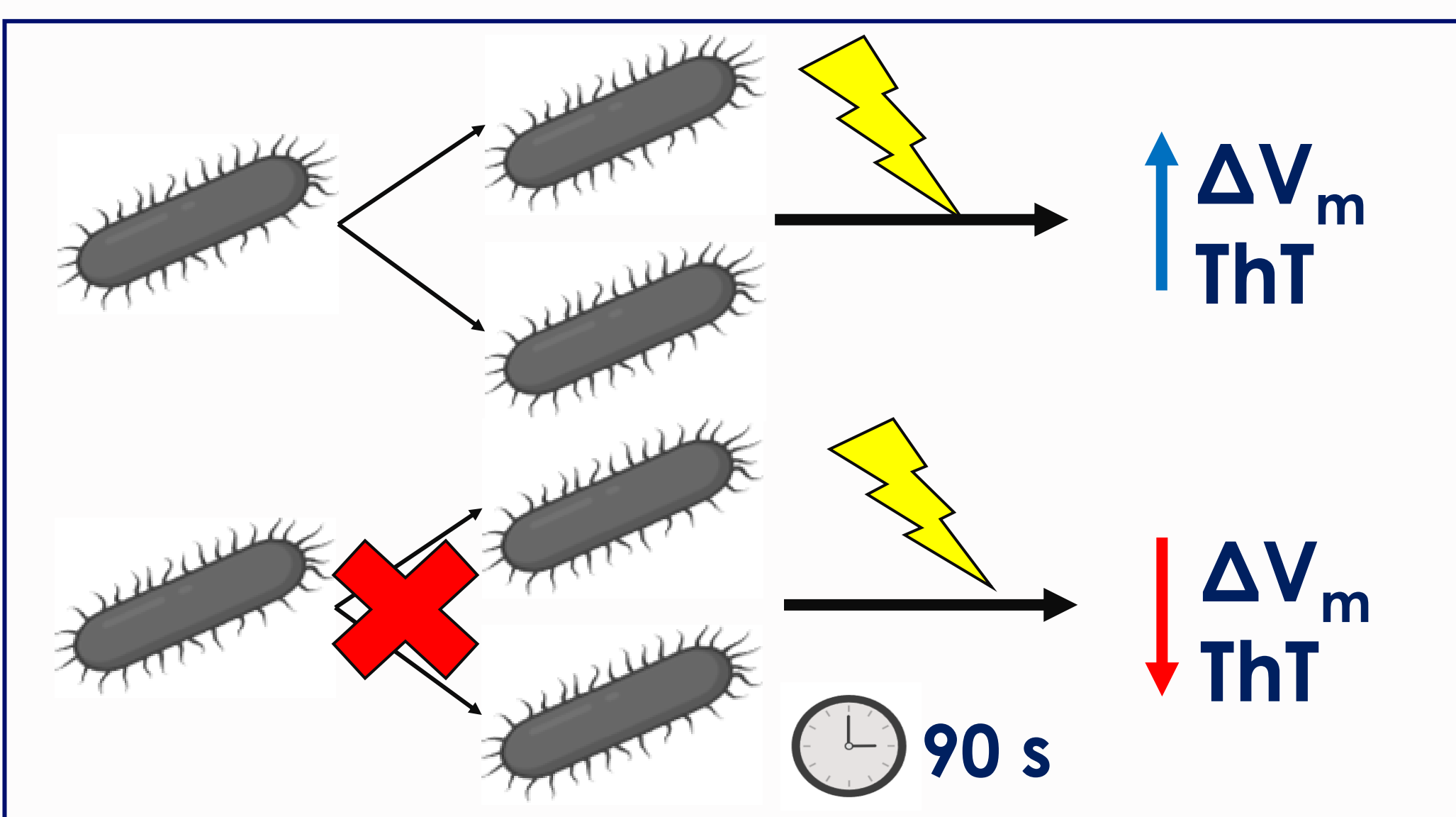
The problem



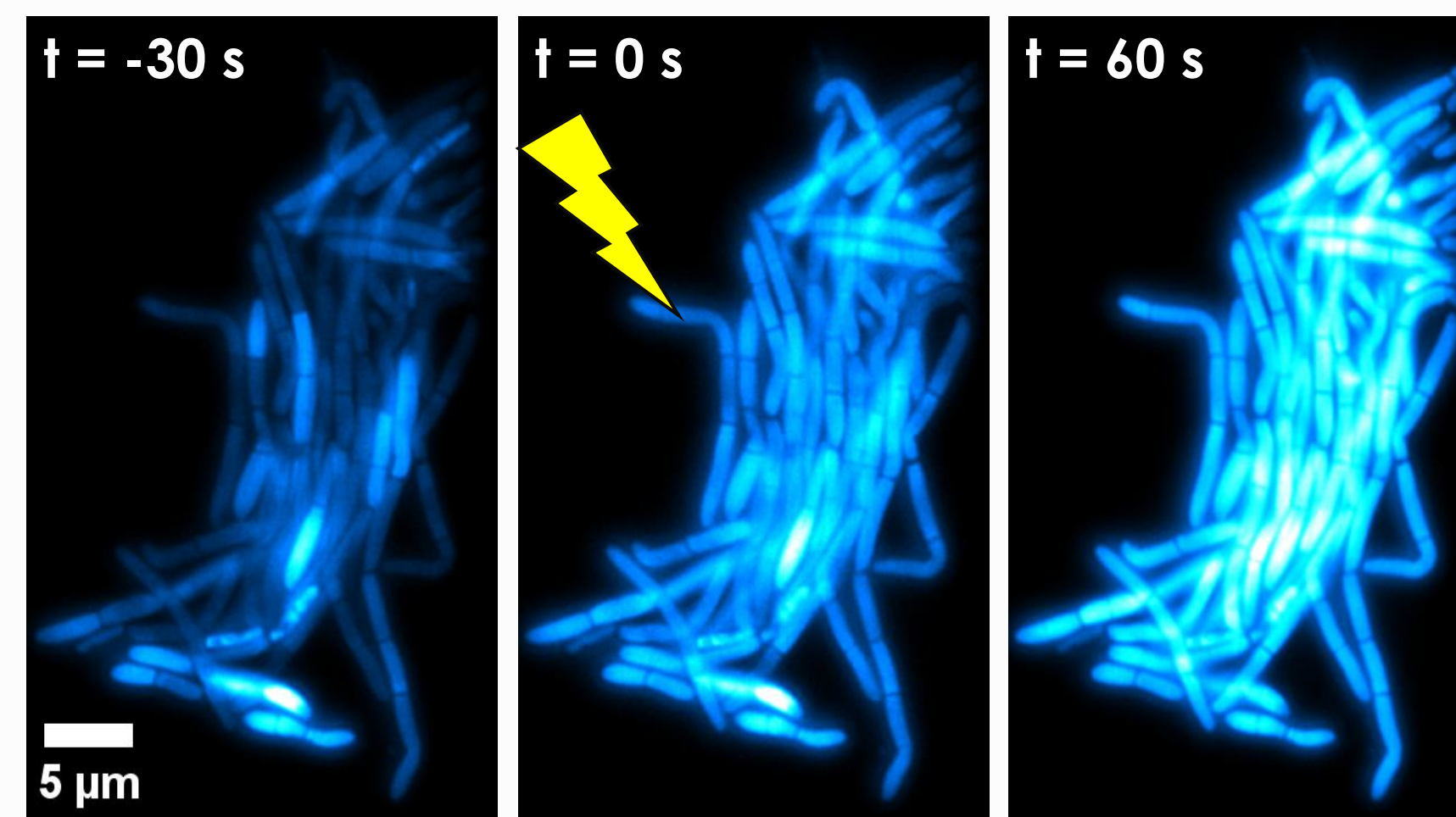
Our aim



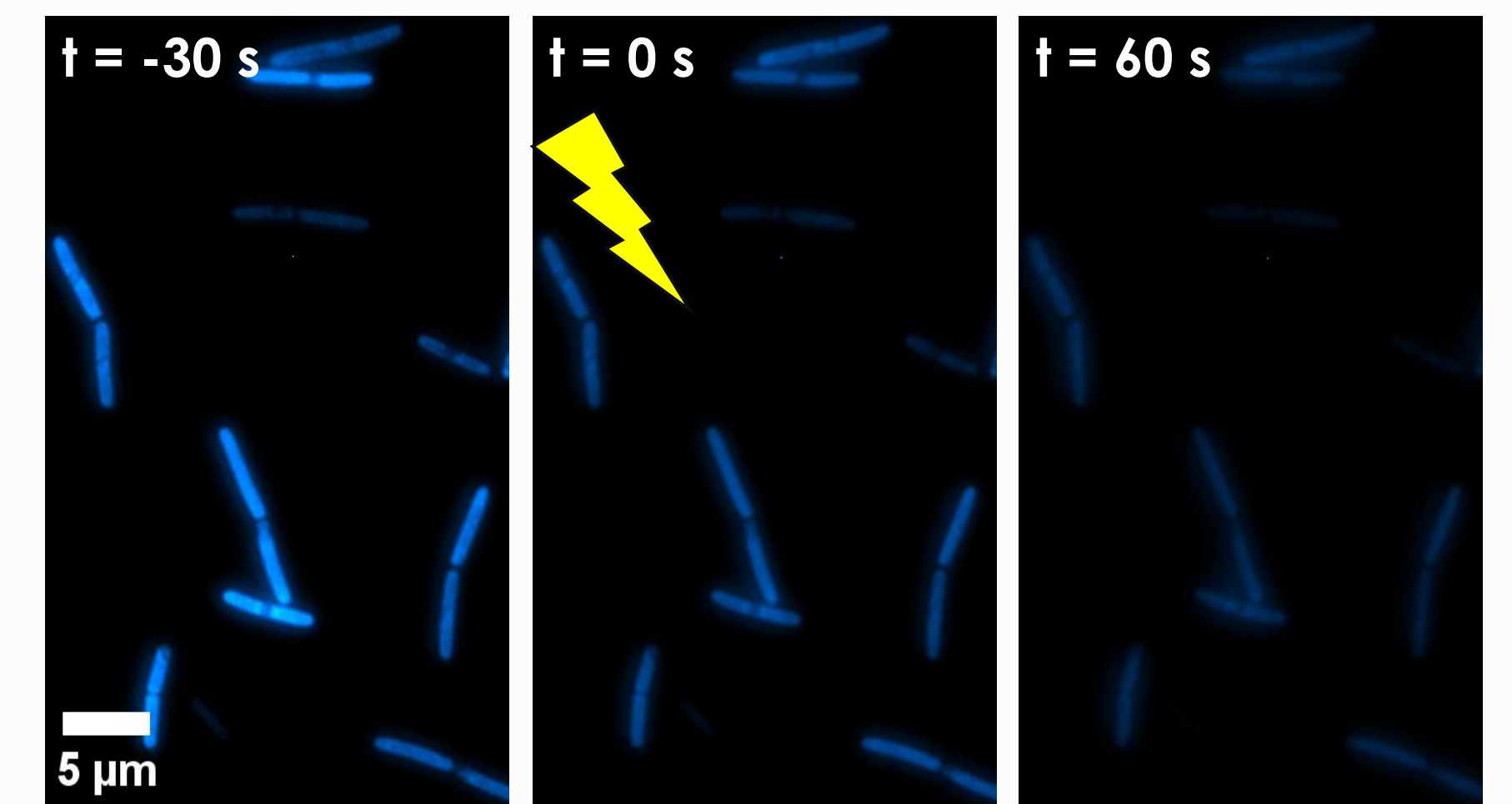
Bioelectrical shock response informs of cell vitality



B. subtilis, no antimicrobial treatment

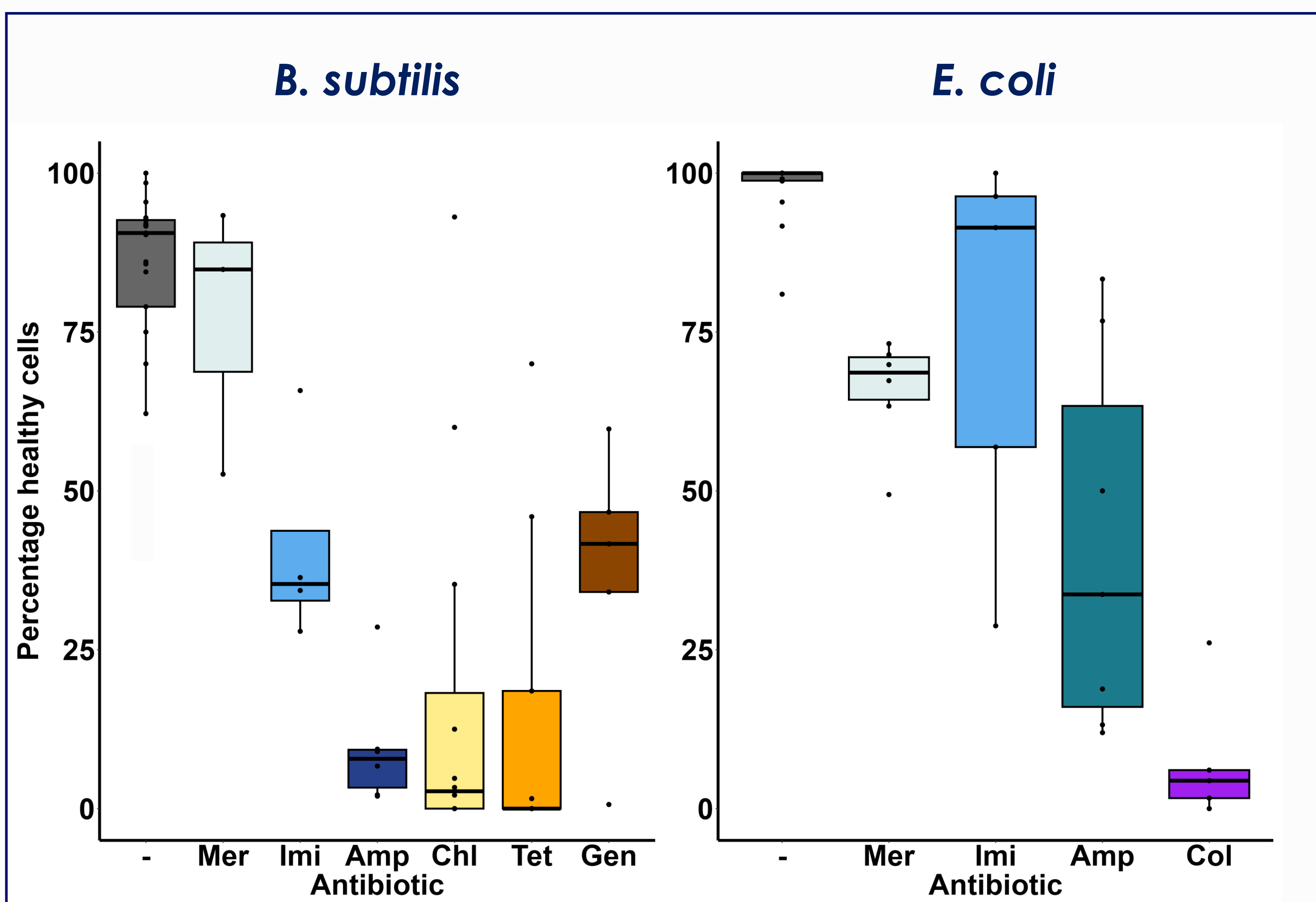


B. subtilis, 0.5 μg/mL vancomycin



(left) Previous work has demonstrated that **proliferative** and **inhibited** microbial cells can be distinguished based on their response to a well-defined **exogenous electrical stimulus**, by **hyperpolarising** and **depolarising**, respectively. By using the fluorescent V_m indicator thioflavin T (ThT), we can quantify this using fluorescence time-lapse microscopy. Examples are given for untreated and vancomycin-treated (right) *B. subtilis*, strain 168, displaying the change in signal.

1 hour exposure sufficient to distinguish treated and untreated populations

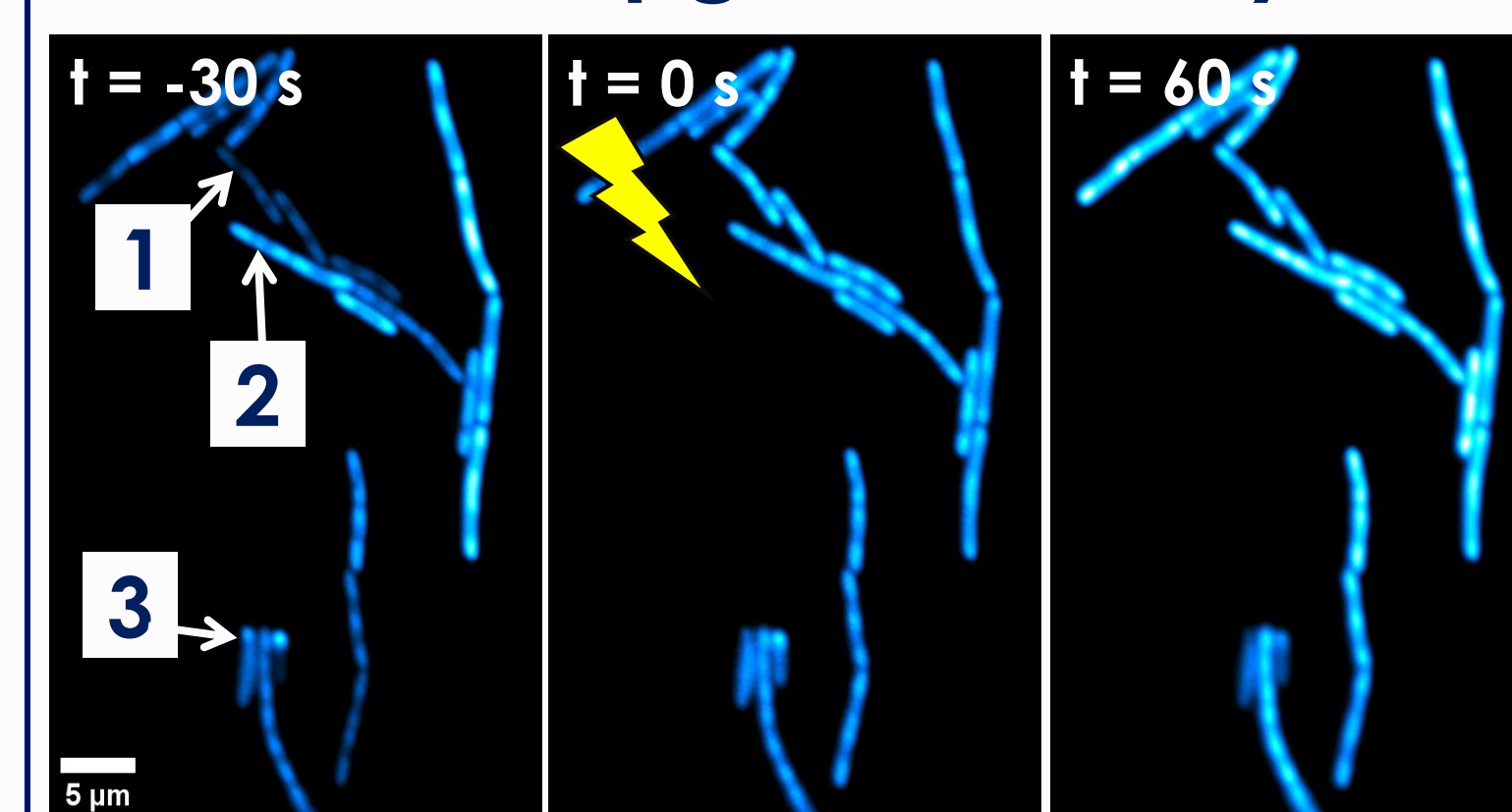


B. subtilis 168 and *E. coli* ATCC25922 were exposed to antibiotics for one hour, at **minimal inhibitory concentrations** determined via broth microdilution. Their responses to electrical stimulation were quantified by measuring the **greatest absolute change** in ThT fluorescence compared to before electrical stimulation.

The proportion of healthy cells was then computed on a per-replicate basis by calculating the percentage of time series which showed a **positive absolute change**. In *B. subtilis*, all antimicrobial treatments produce a clear reduction in healthy cells according to our metric. In *E. coli*, however, our experiments show **more variability**, nonetheless appearing to have a measurable impact on shock response. An expansion of the *E. coli* dataset is in progress. In addition, work is ongoing regarding the development of **further methods of quantification** to maximise test accuracy.

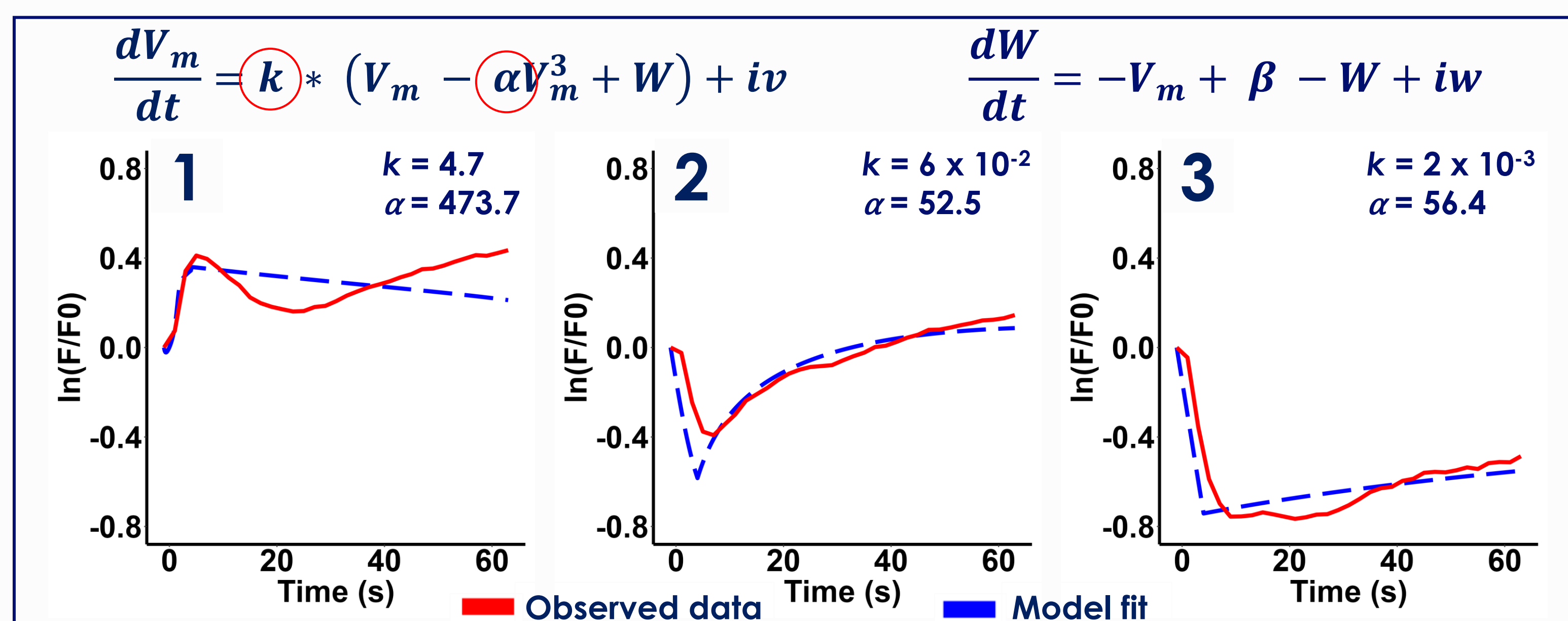
FitzHugh-Nagumo model allows precise quantification of vitality

B. subtilis, 8 μg/mL tetracycline



We have made further developments to the bacterial **FitzHugh-Nagumo (FHN)** model, allowing us to accurately capture the post-shock dynamics of bacterial cells (left), extracting the k and α parameters for further analysis (below).

This presents a great opportunity for an **accurate quantification** of susceptibility beyond a "living"/"dead" binary.



Conclusions & future work

Our method appears **robust across a diverse selection of antibiotics**, including highly clinically relevant antimicrobials, such as **meropenem** and **colistin**. In conjunction with the **granular quantification** of cell vitality outlined above, we demonstrate the great potential of this electrophysiological technique.

We also intend to extend validation to other clinically relevant species, such as ***P. aeruginosa*** and ***C. albicans***. Further development of the FHN-based analysis method is also required for **timely processing** of test results.