

# Thermodynamic and kinetic effects on the activity and selectivity of antimicrobial peptides

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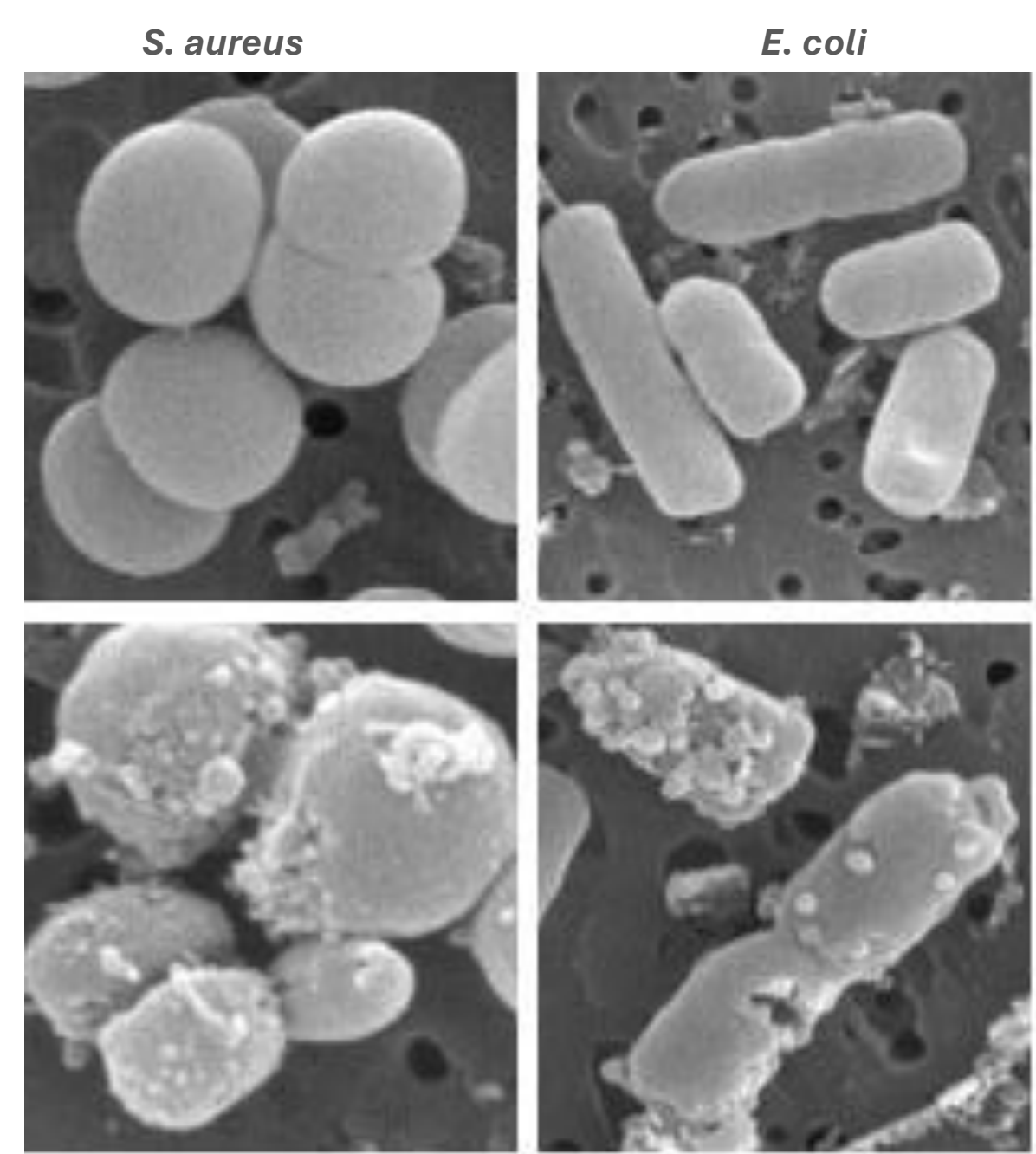
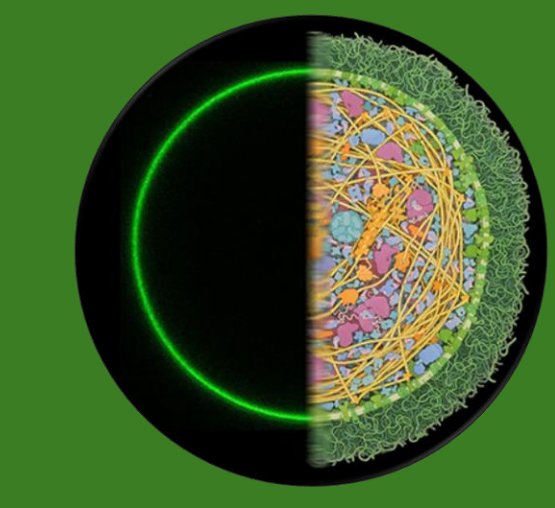
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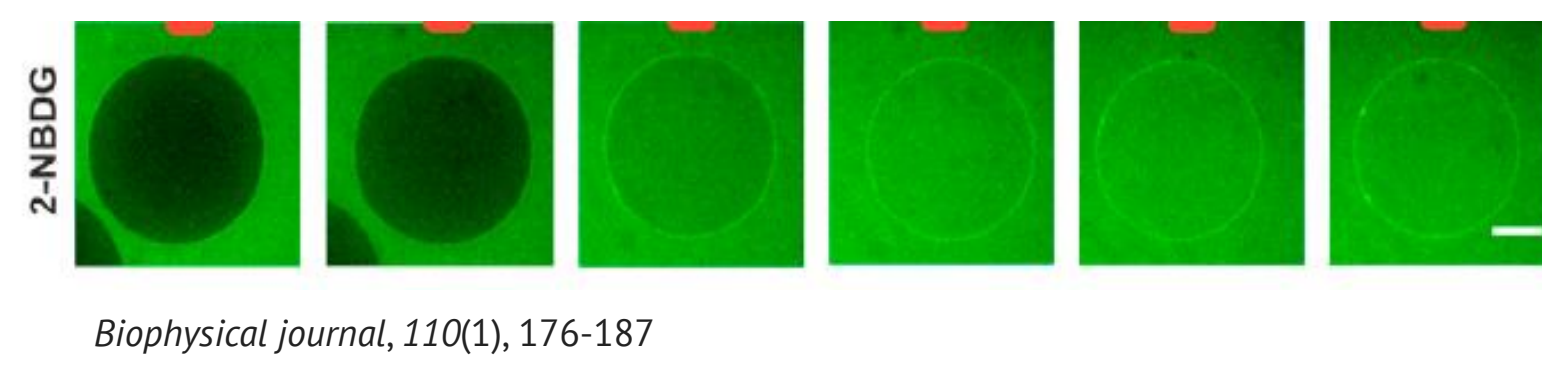
Produced by all organism

Short sequences (10-50 a.a.)

Cationic and amphipatic

Kill pathogens rapidly by making their membrane permeable

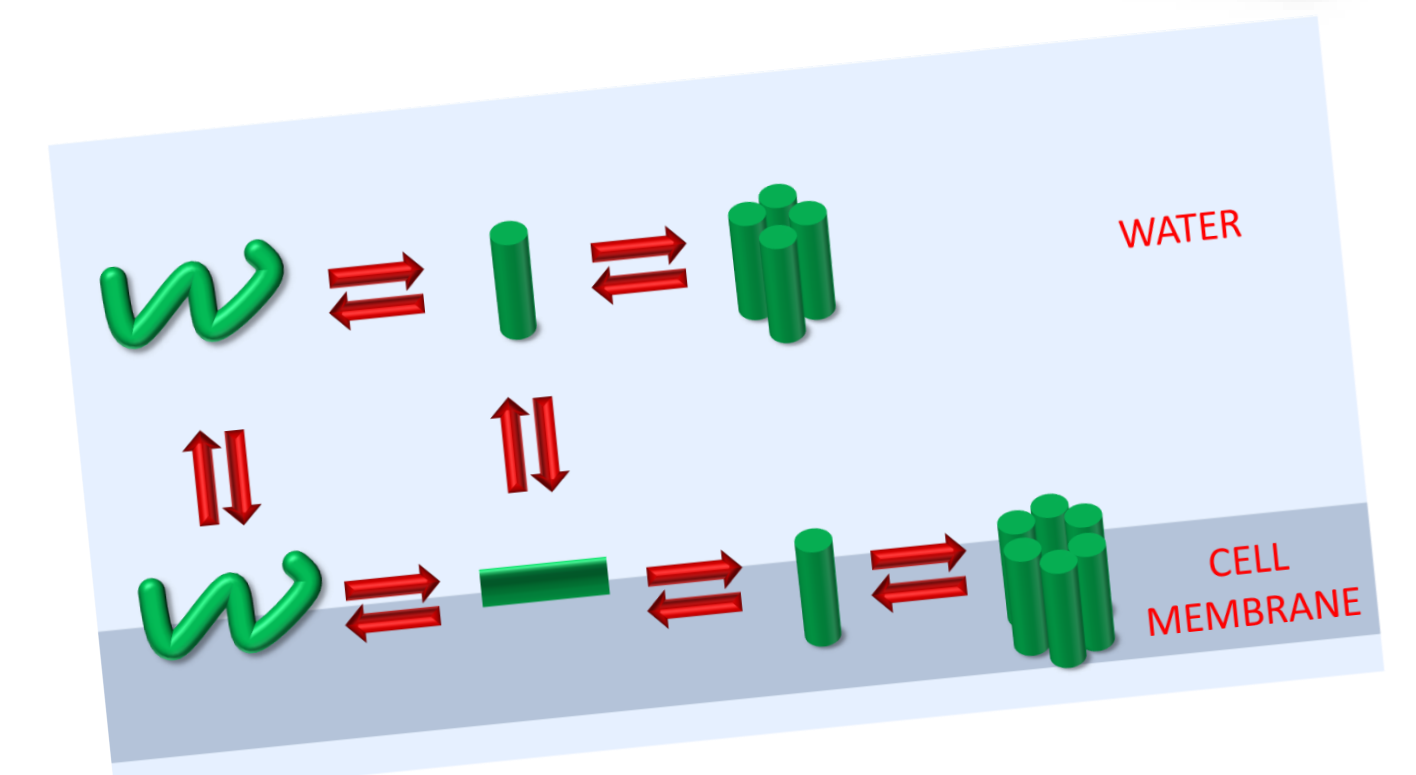
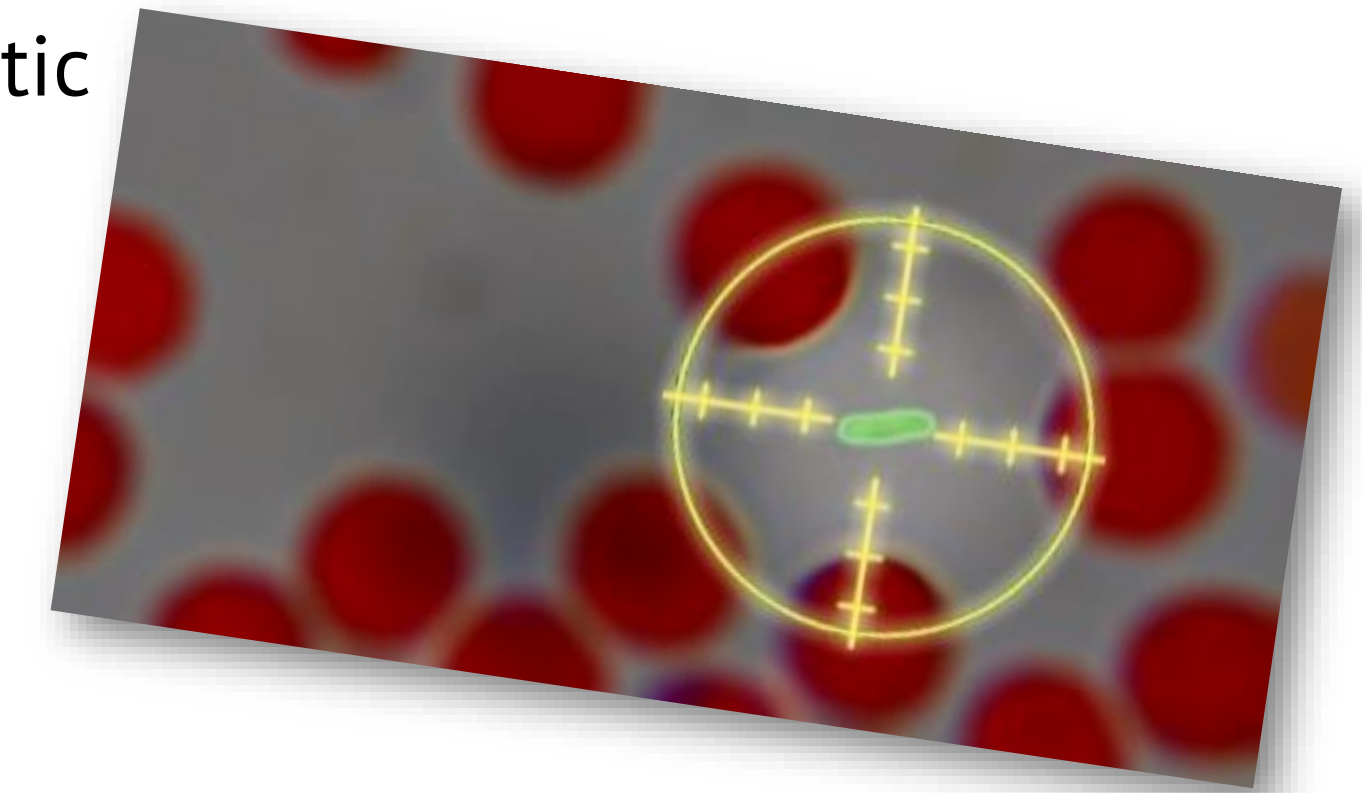
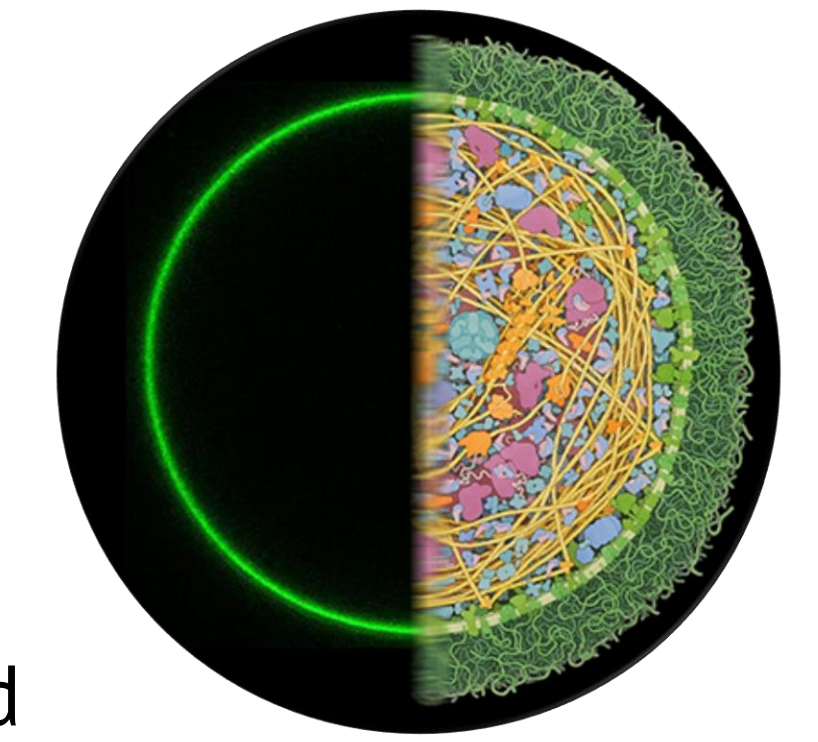
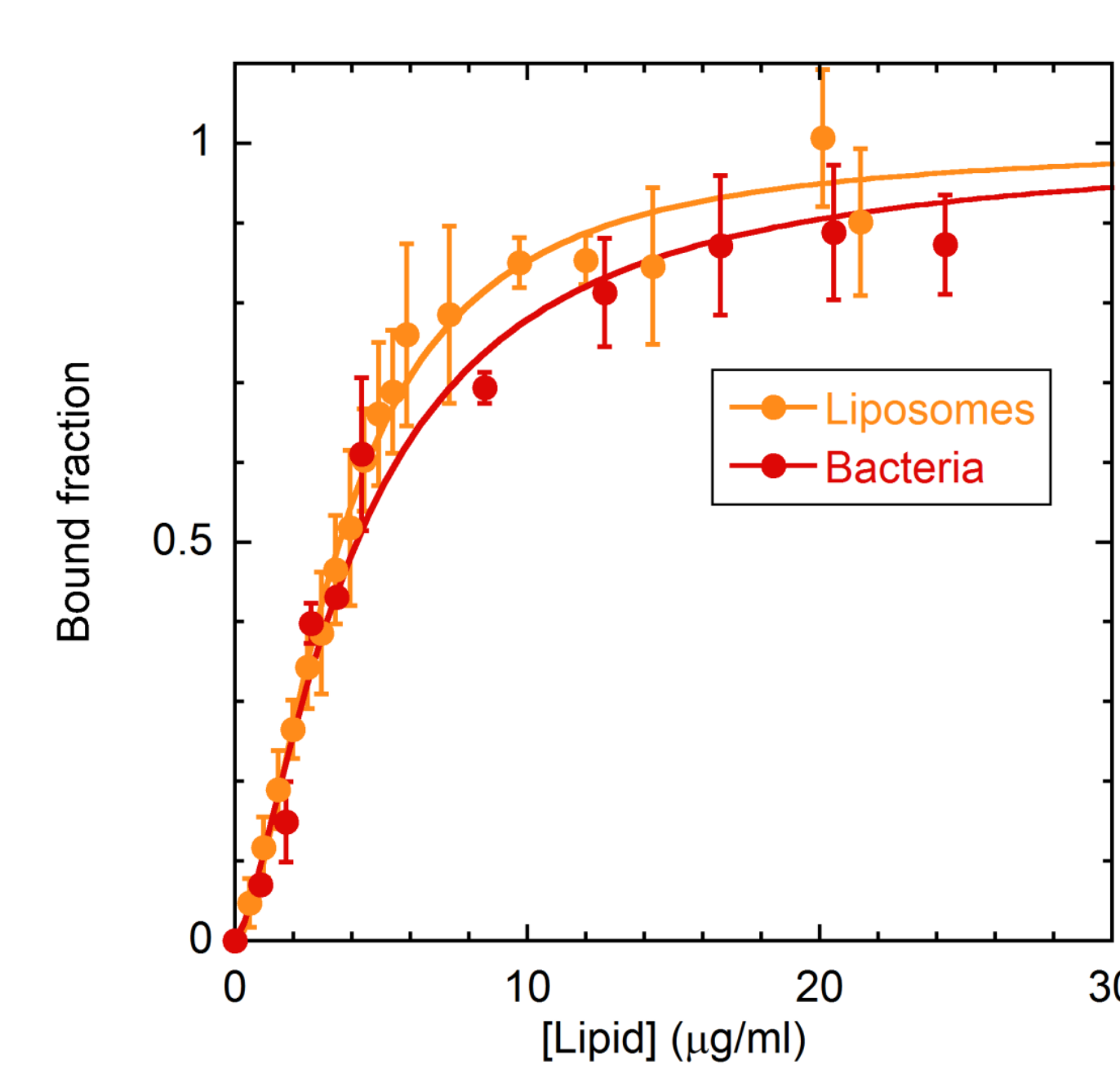
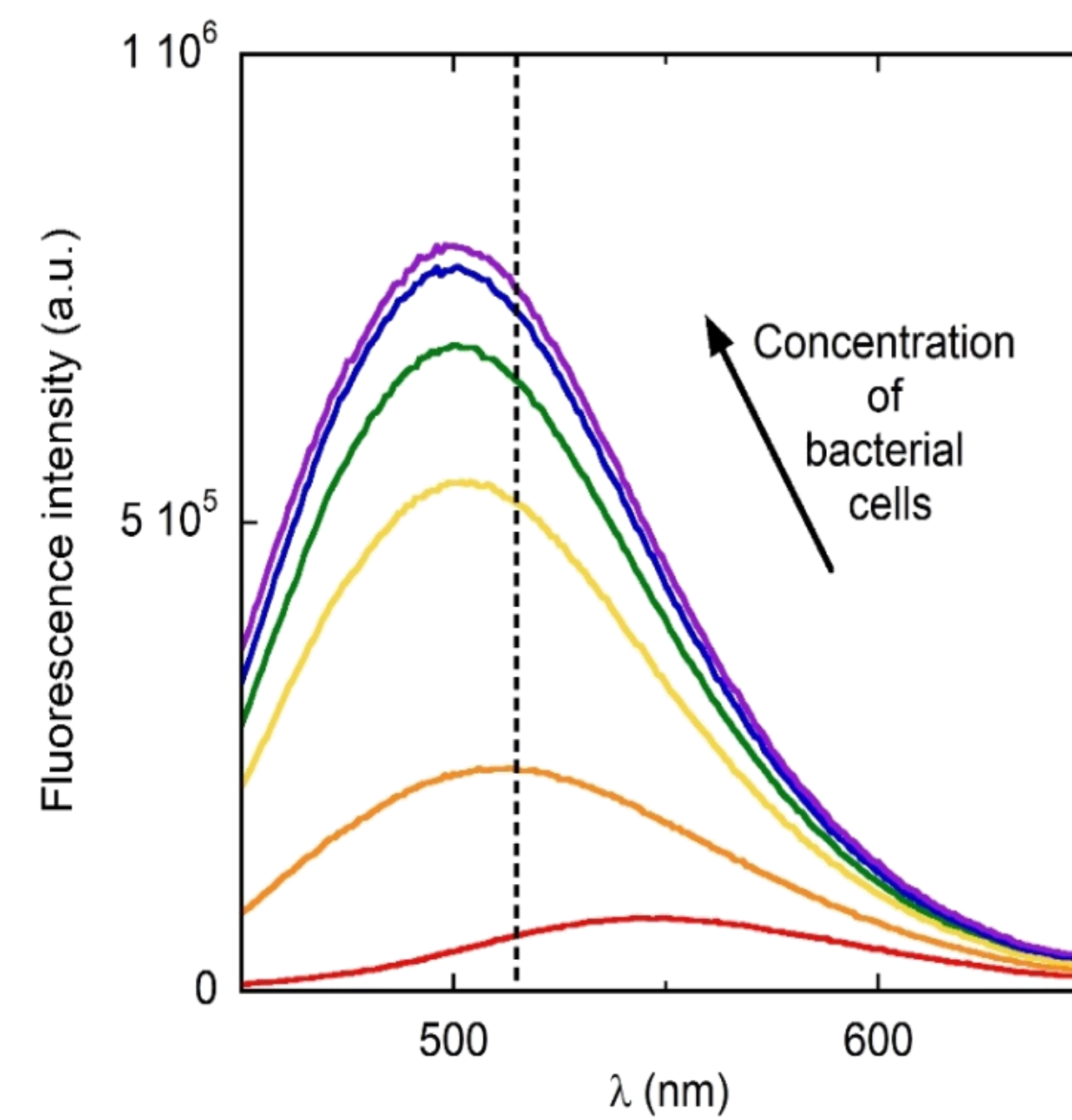
Selective (due to the different composition of bacterial and host membranes)



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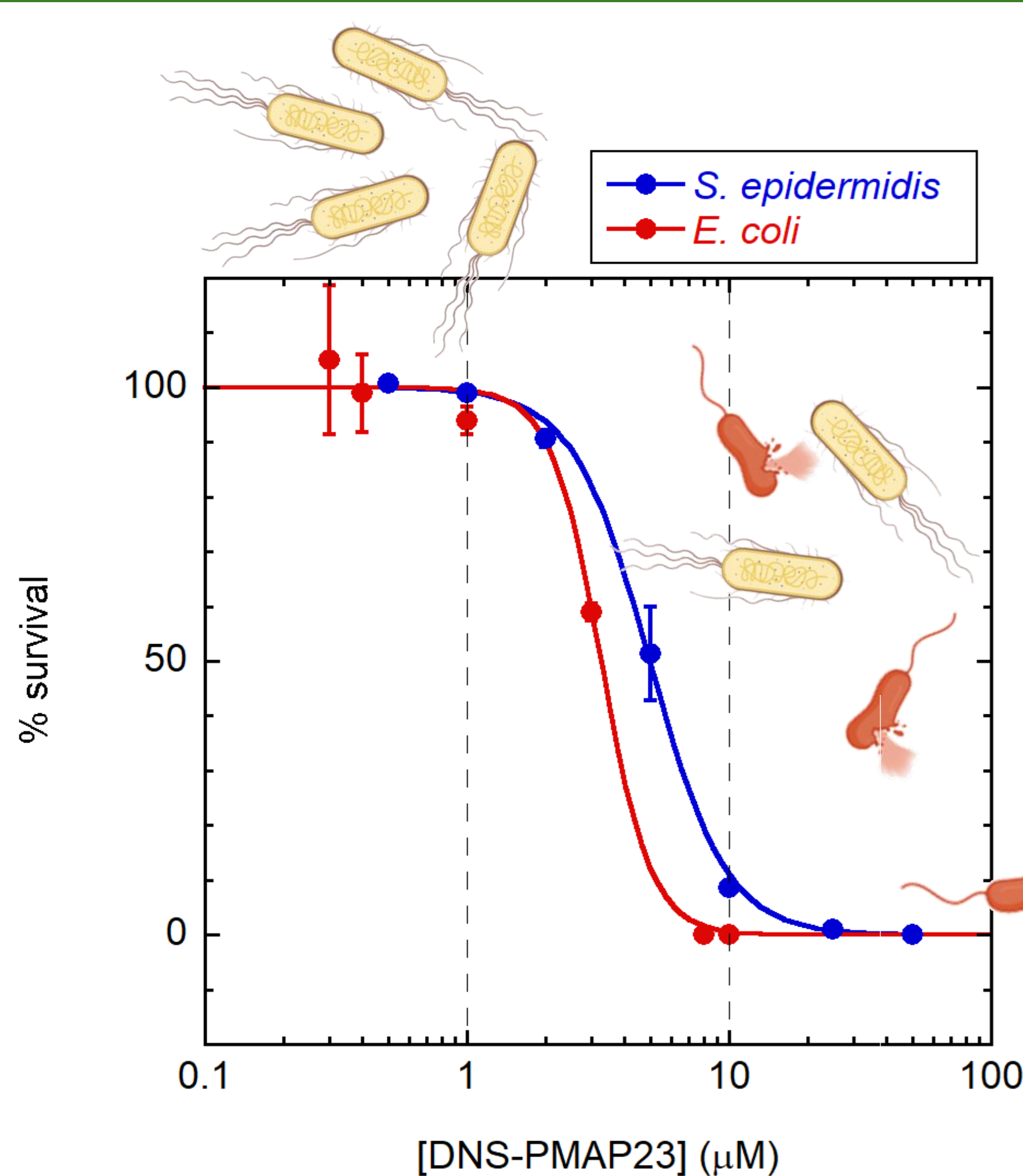
## What have we learned about antimicrobial peptides (AMPs)?

- Liposomes are a good model to study AMPs
- Spectroscopic methods can be applied to live bacterial cells
- The active concentration depends on cell density (both for bacteria and host cells)
- Microbiological assays should be modified to better represent realistic conditions

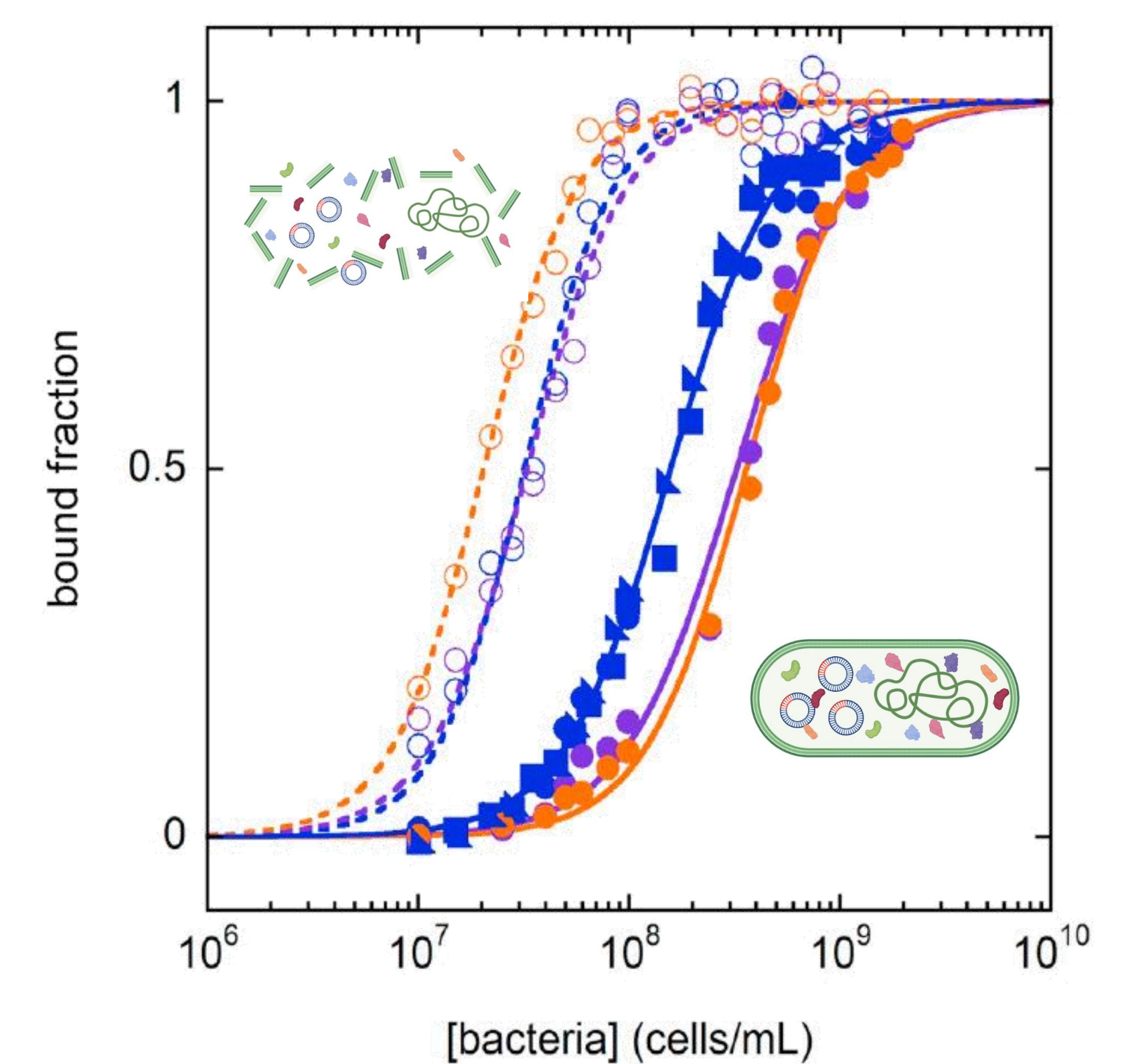
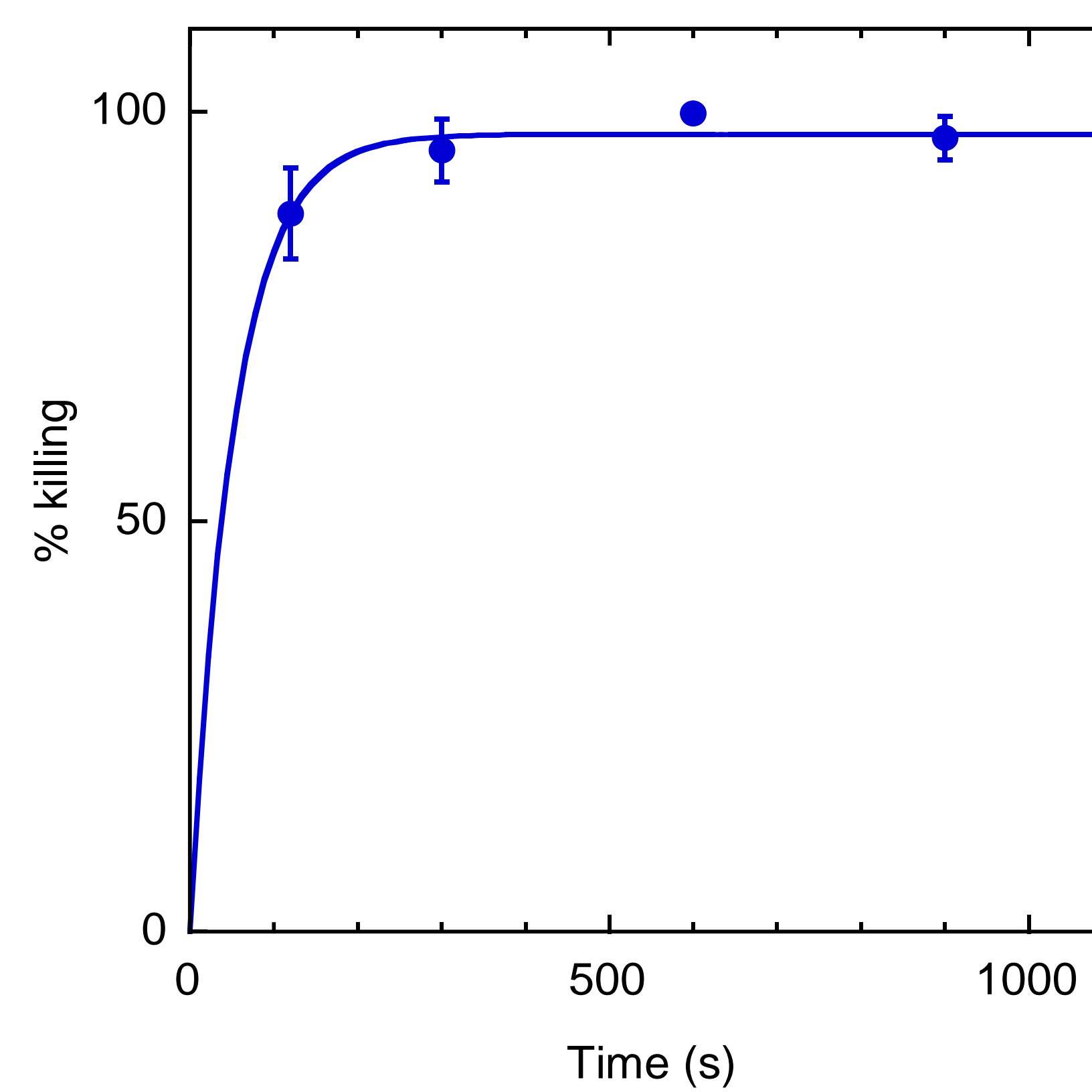


## Open questions

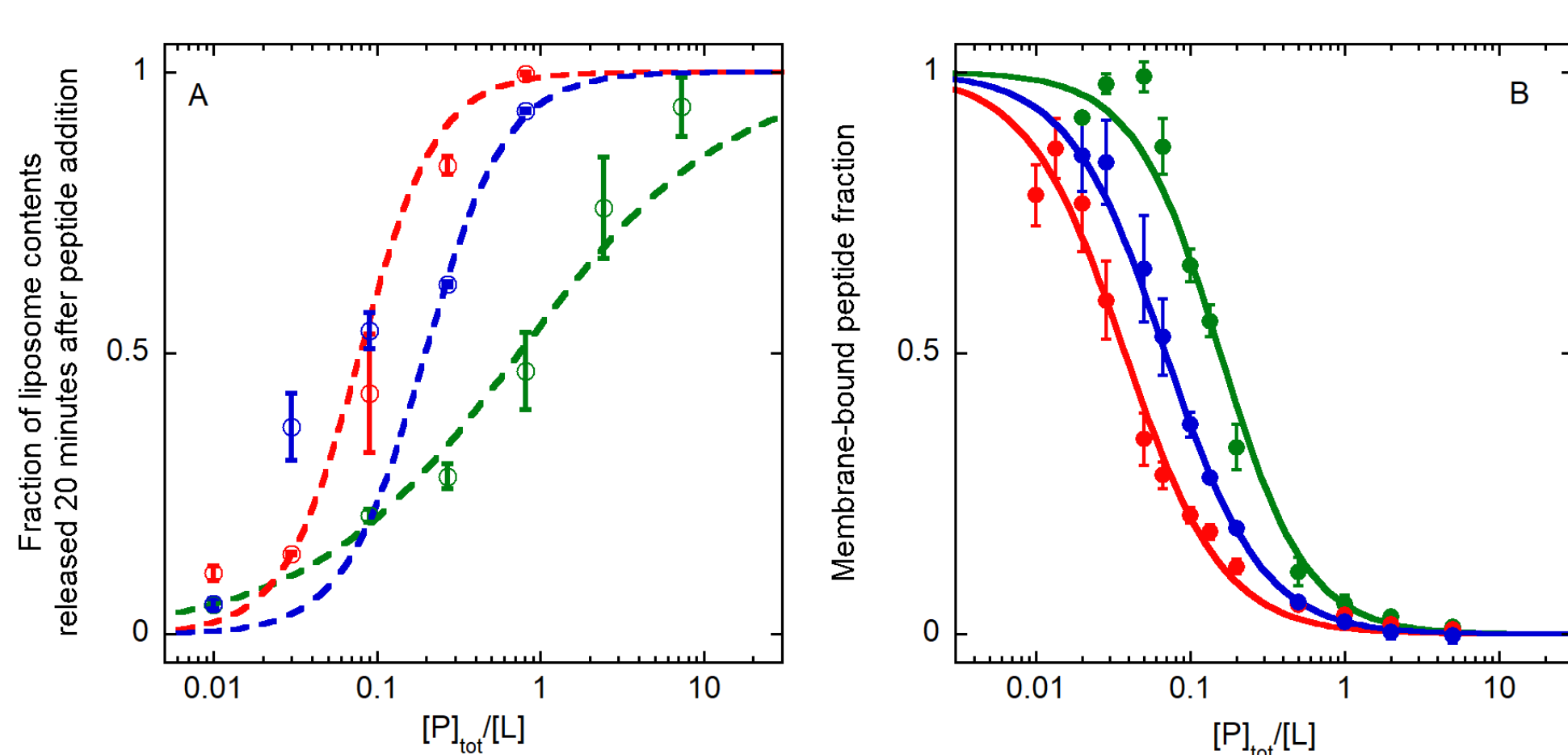
### What is the sequence of events involved in bacterial killing



### What is the origin of the mutant selection window (MSW)

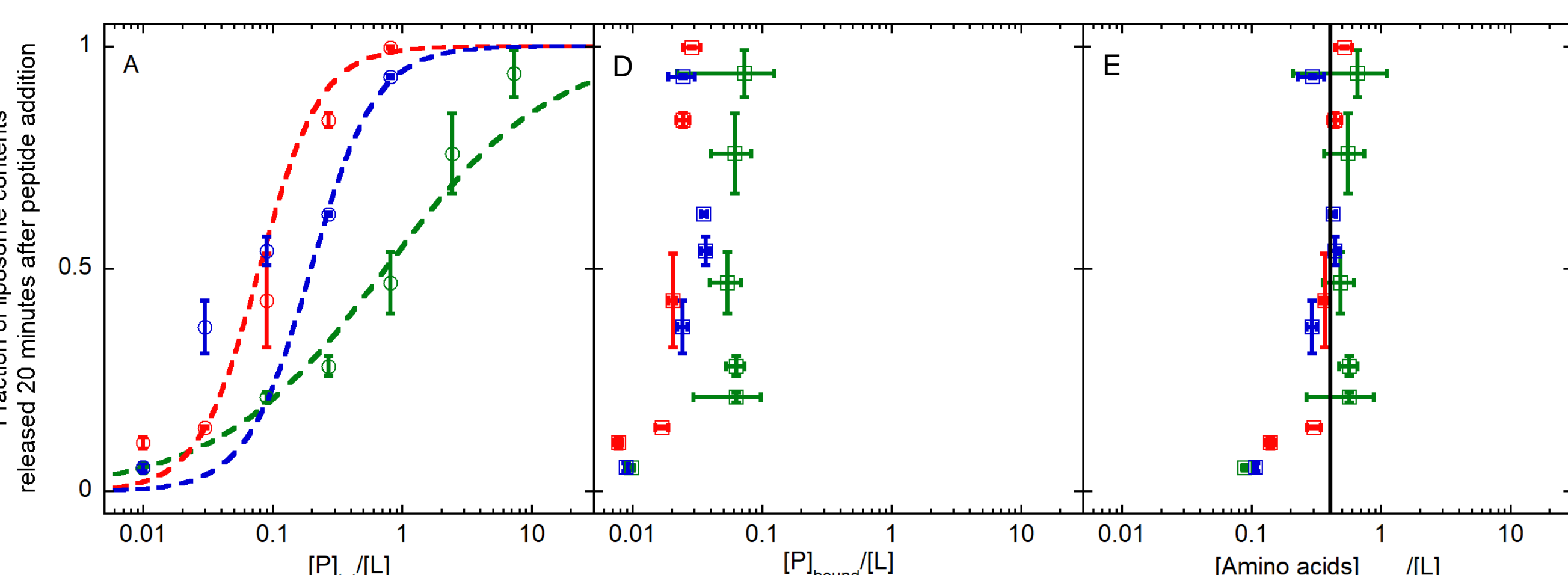


## Mutant selection window: the role of water-membrane partition

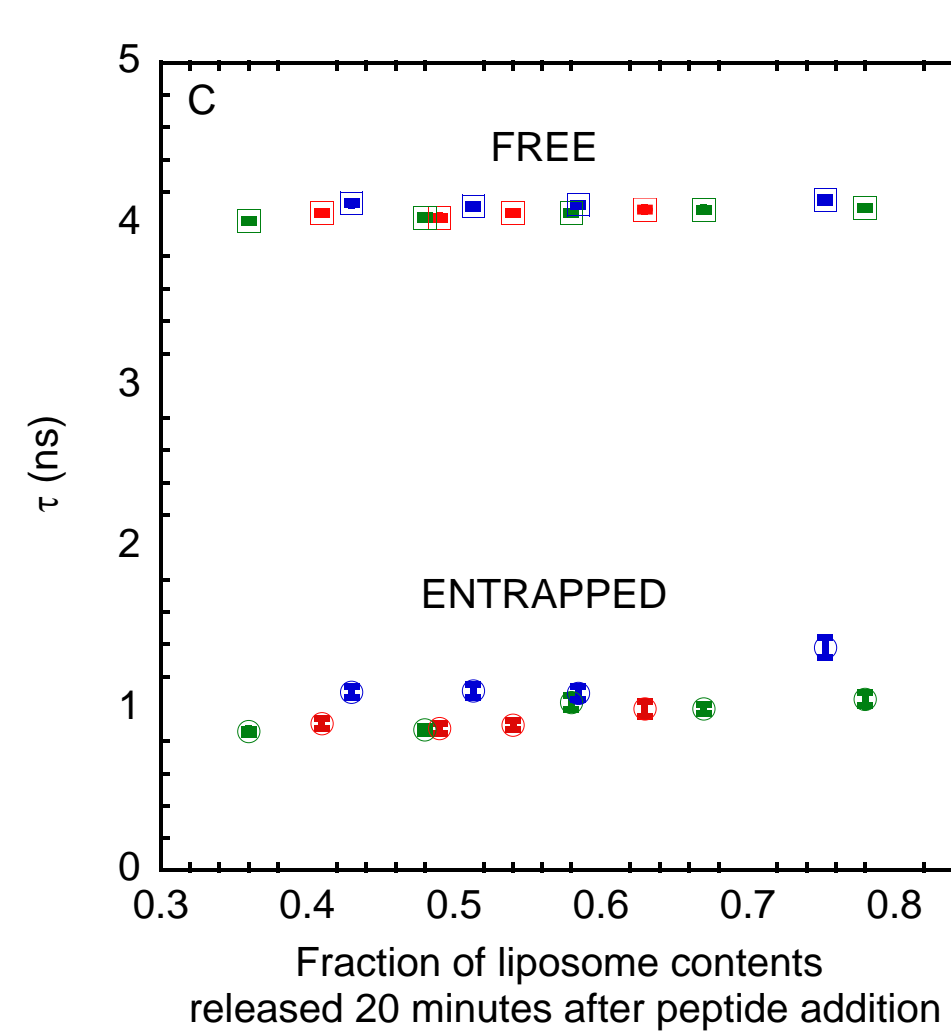


- We followed spectroscopically the membrane-perturbing activity of different antimicrobial peptides in model membranes and we found the same heterogeneous response observed in bacteria (A)
- In an extremely wide range of  $[P]_{tot}/[L]$  values ( $10^{-2}$  to 10) only a fraction of the liposomes released their contents: these peptides have an "all or none" mechanism of action (C)

- By measuring the membrane-bound peptide fraction at different lipid concentrations (B), we reported the curves (A) as a function of the peptide actually bound to the membranes (D,E)

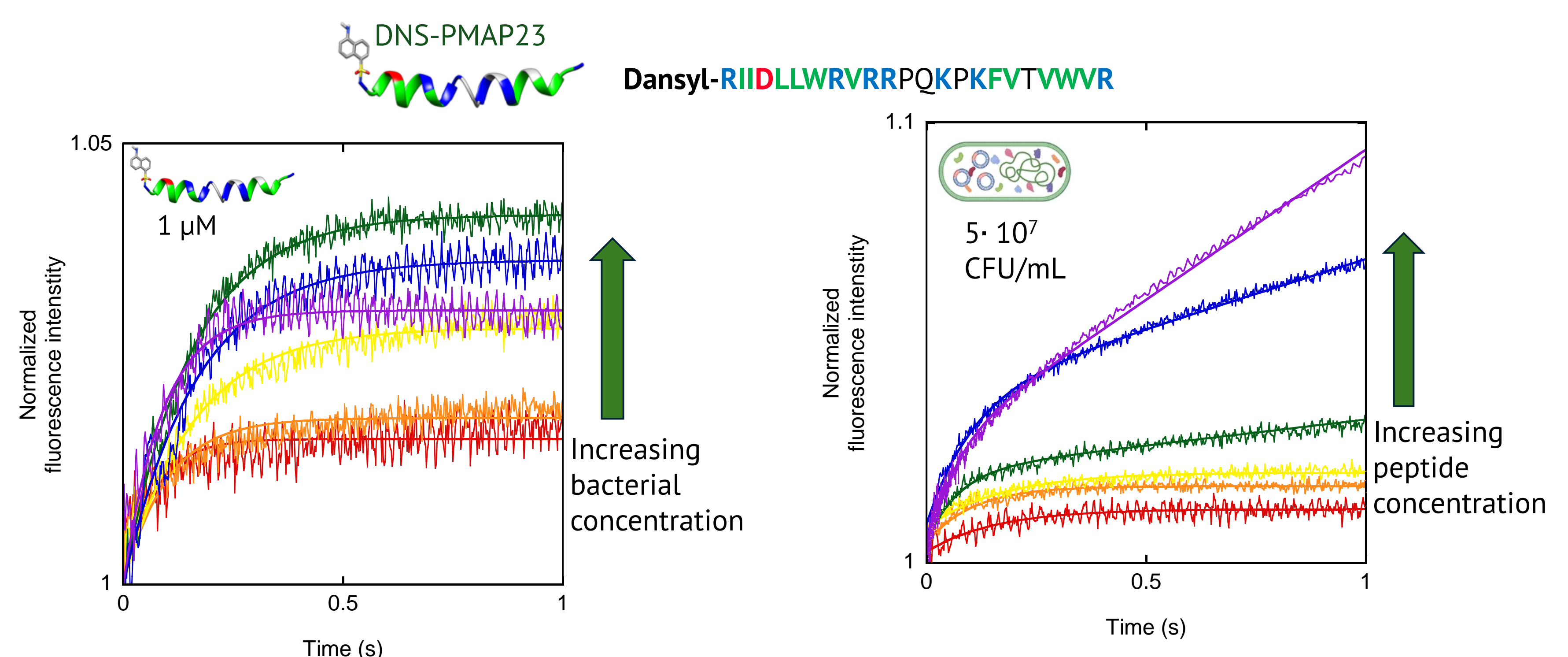


|           | Sequence                         | AA <sup>a</sup> | Q <sup>b</sup> |
|-----------|----------------------------------|-----------------|----------------|
| Novicidin | KNLRRIRKGIHIKKYF-NH <sub>2</sub> | 18              | +8             |
| P9-4      | KWRRWIRWL-NH <sub>2</sub>        | 9               | +5             |
| Sub3      | RRWRIVRVR-NH <sub>2</sub>        | 12              | +7             |

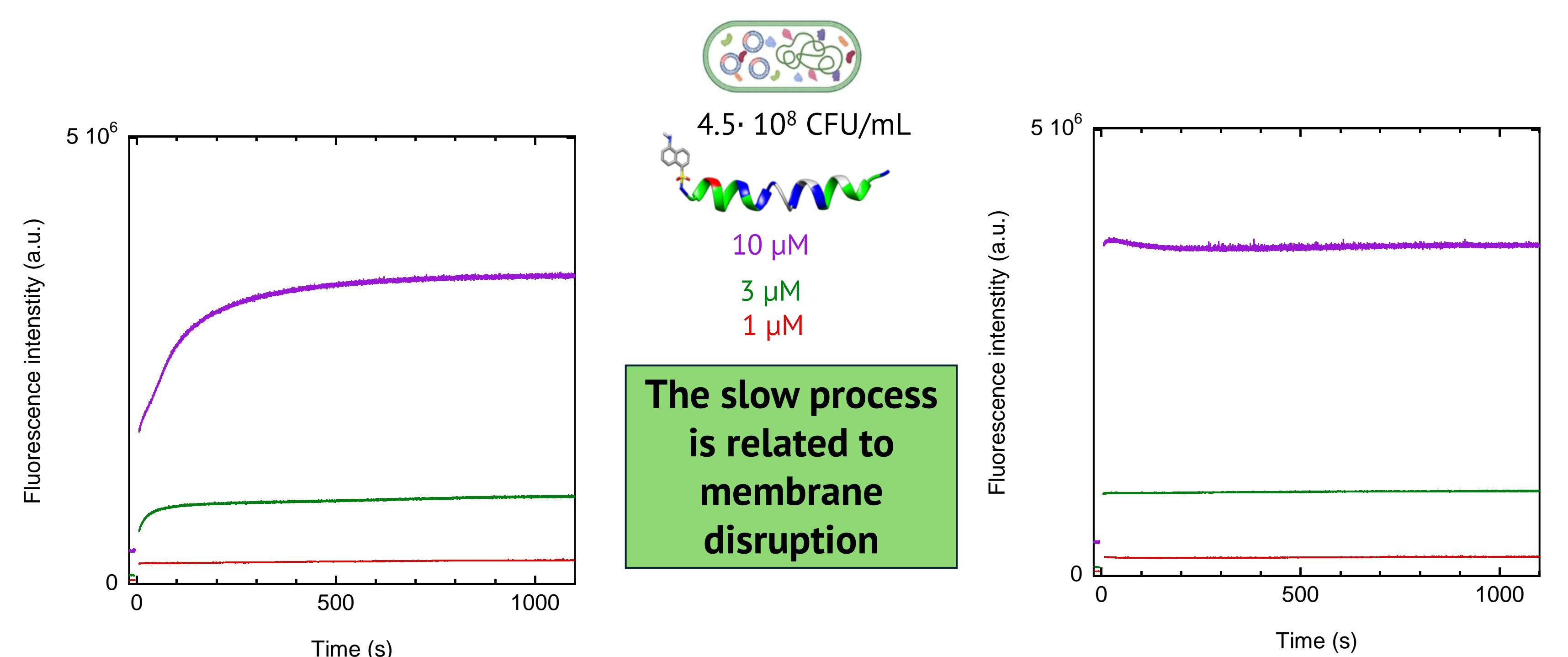


- Different peptides show the same threshold necessary to form pores: steric mechanism of action
- Heterogeneous response determined only by the variation of the fraction of membrane-bound peptides

## Sequence of events in bacterial killing: kinetics of peptide/cell interaction



- Cell-binding is complete in less than 1 s
- Additional slower process only at peptide concentration causing killing



The slow process is related to membrane disruption