



# Probing the membranolytic activity of novel quaternary ammonium compounds using atomic force microscopy



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# Quaternary ammonium compounds (QACs)

- ✓ one or more positively charged nitrogen atom(s) with four bonds
- ✓ amphiphile molecules (cationic surfactants)
- ✓ potent antimicrobial agents

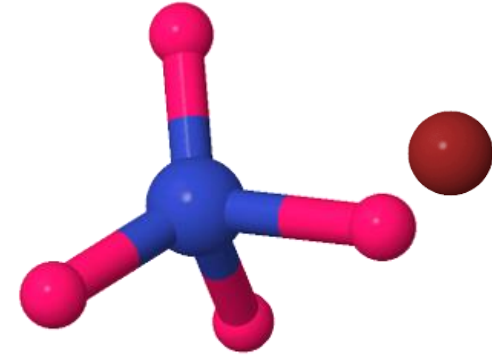


Figure 1. General structure of QAC

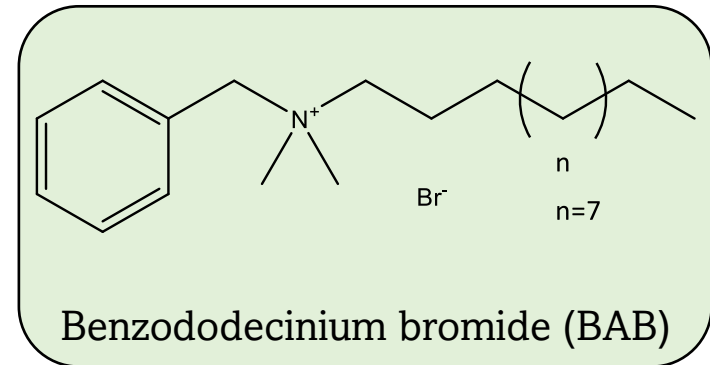
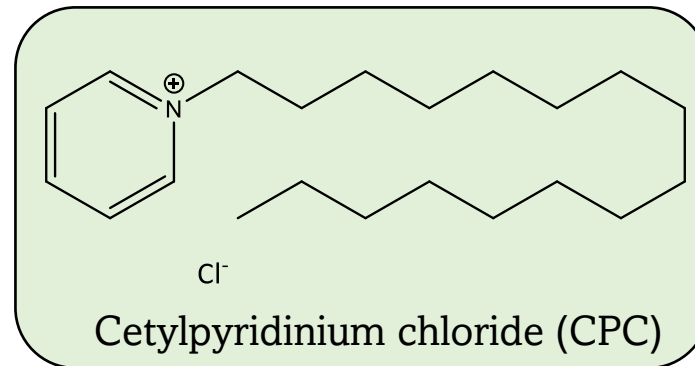
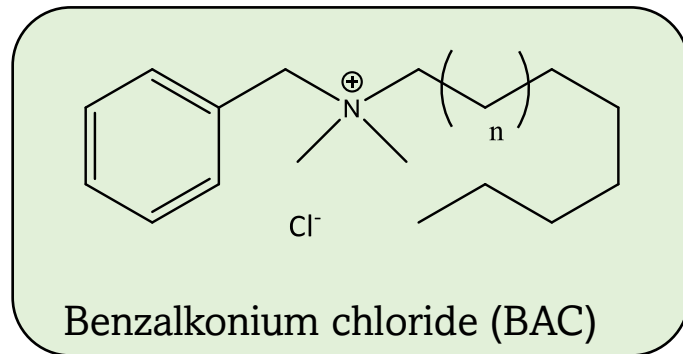
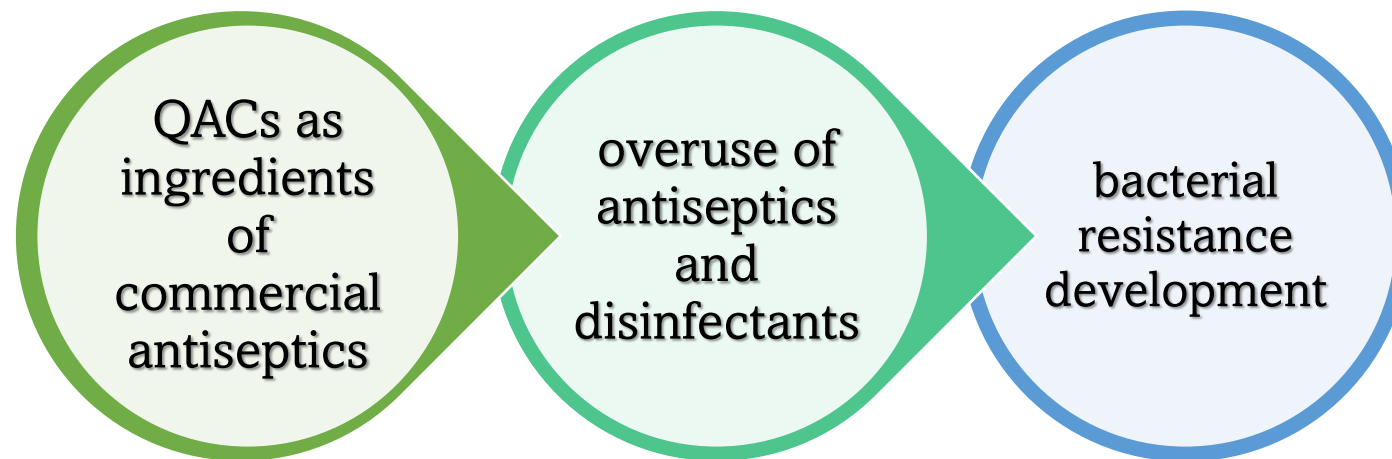
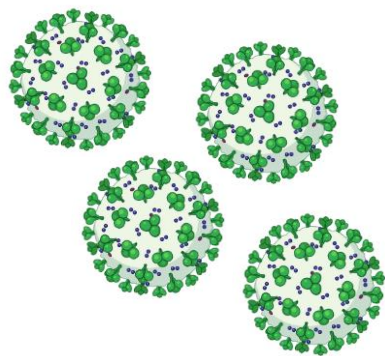


Figure 2. Common commercial QACs



- ✓ synthesis of new QAC variants with improved biological activity
- ✓ natural scaffolds as precursors for novel QACs synthesis

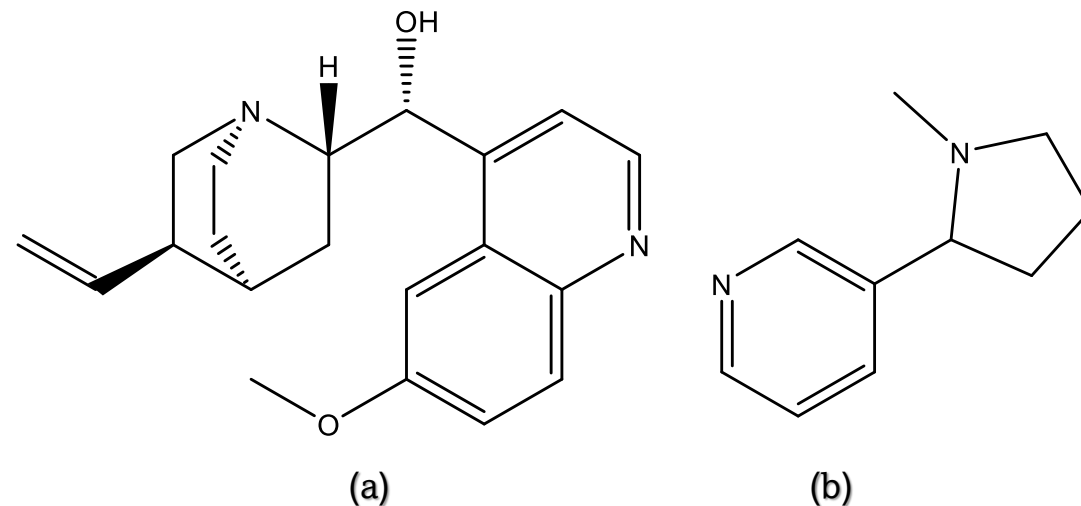


Figure 3. Structures of quinine (a) and nicotine (b)

...previously in literature

### Biological activity of natural product derived QACs

- ✓ most potent QACs have alkyl chains with 12-14 C atoms

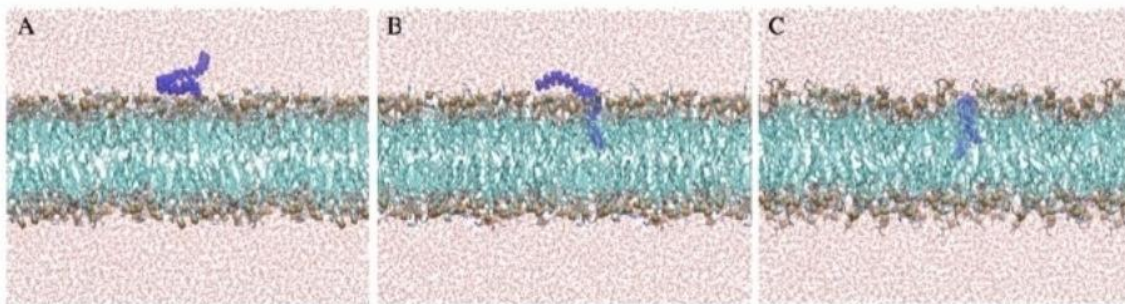
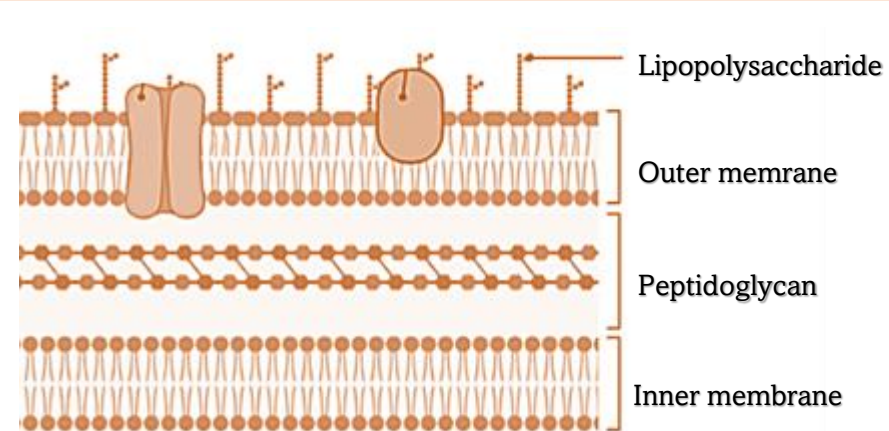
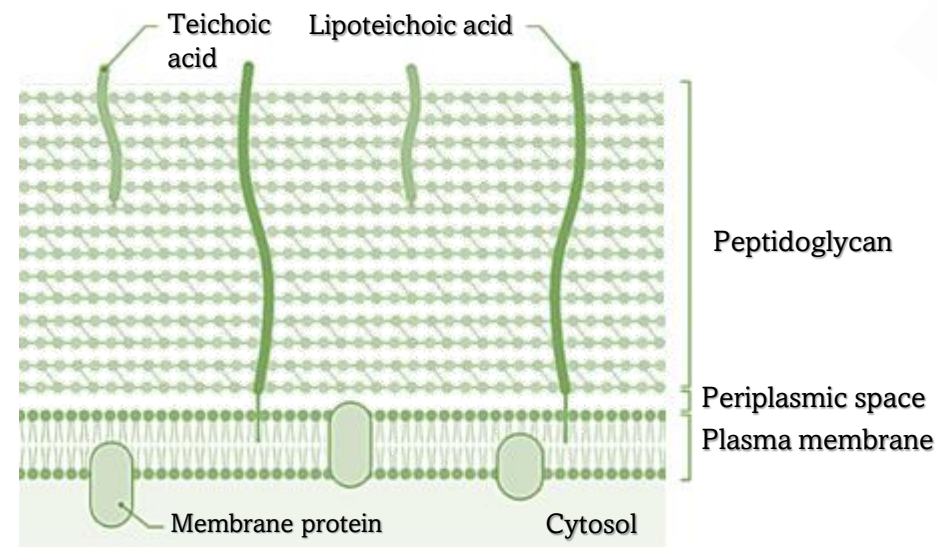


Figure 4. Integration of QAC into modelled bacterial membrane

### Gram-negative bacterial membrane



### Gram-positive bacterial membrane



[2] Minbiole, K. P.C.; et al., From antimicrobial activity to mechanism of resistance: the multifaceted role of simple quaternary ammonium compounds in bacterial eradication. *Tetrahedron*, 72(25), 3559–3566.

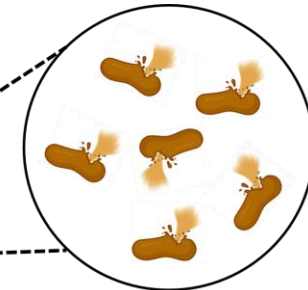
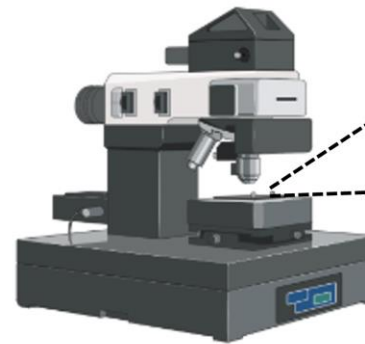
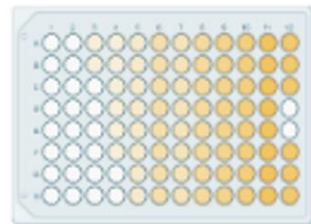
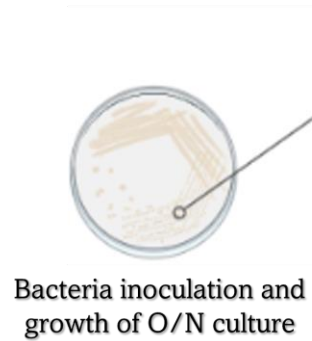
[3] Alkhalifa, S., Jennings, M., Granata, D., Klein, M., Wuest, W. M., Minbiole, K., & Carnevale, V. (2019). Analysis of the Destabilization of Bacterial Membranes by Quaternary Ammonium Compounds: A Combined Experimental and Computational Study. *ChemBioChem*.



Synthesis of novel quaternary ammonium compounds (QACs)

Investigation of biological activity

Investigation of bacterial resistance mechanisms and **mode of action**



## ...our previous investigation

① naturally occurring structure

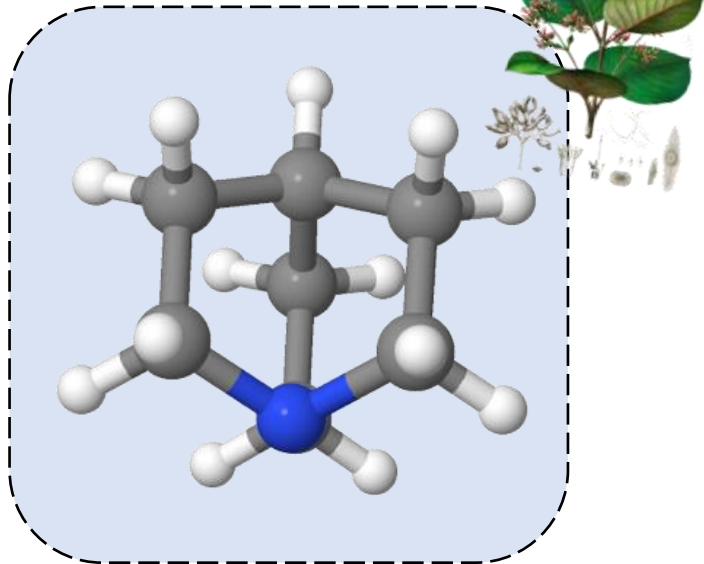


Figure 5. Structure of quinuclidine

② 3-substituted derivative

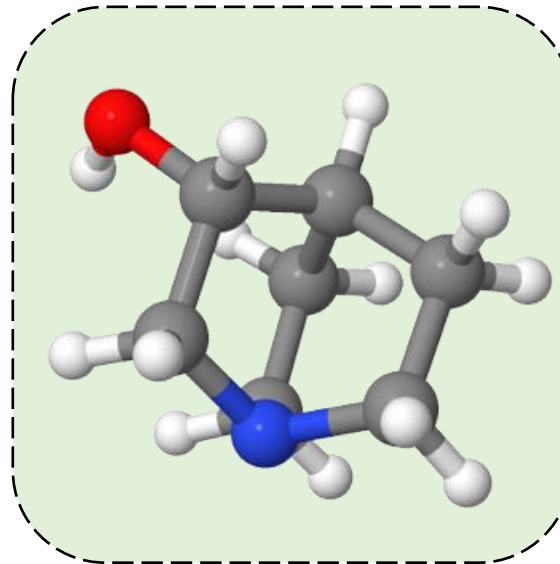
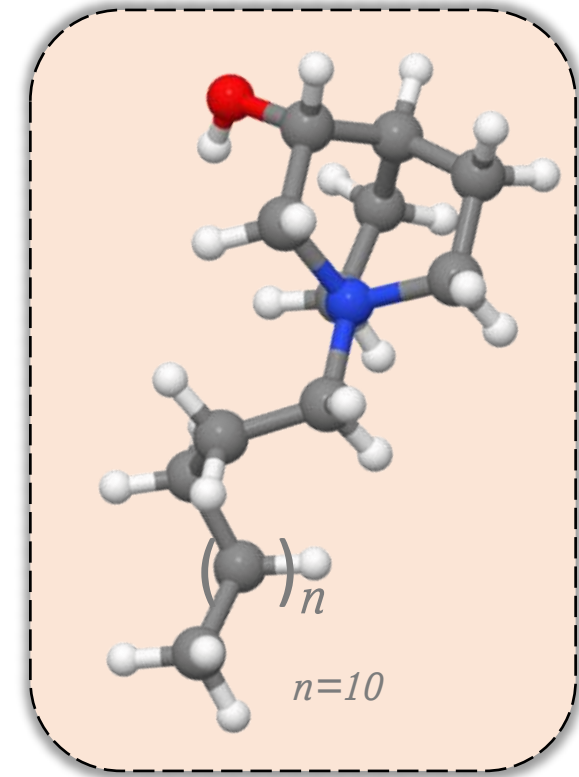


Figure 6. Precursor structure of 3-substituted quinuclidine

③ new QAC derivatives

Figure 7. Structure of **QOH-14**, most potent synthesized QAC

## ...our previous investigation

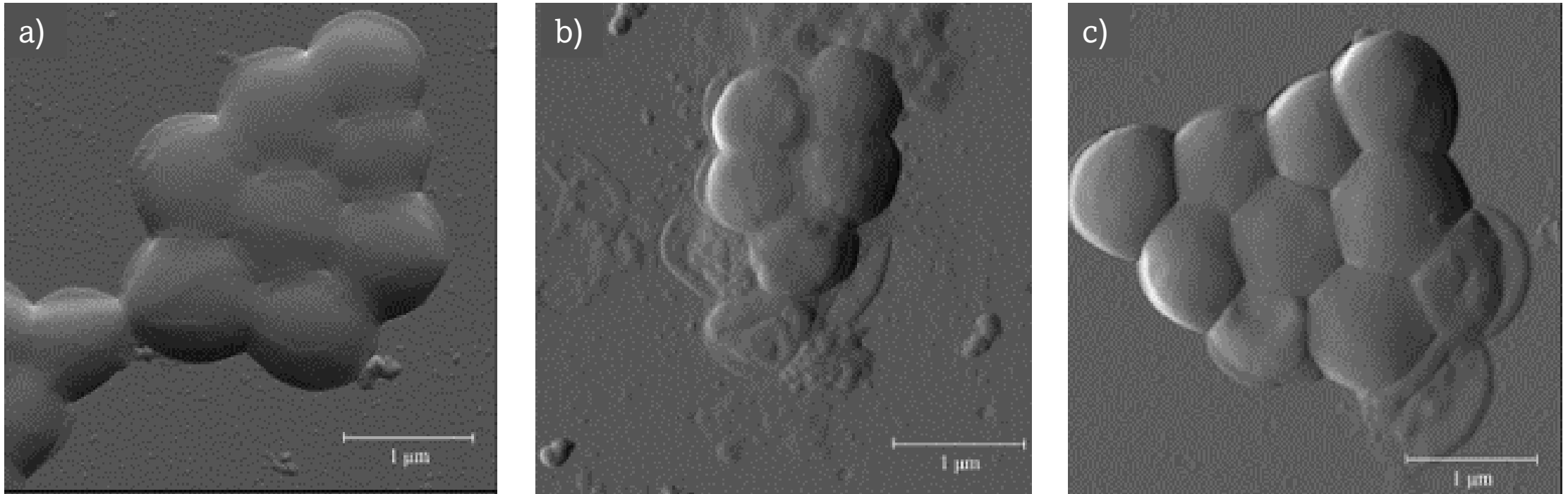


Figure 8 a) – c). AFM deflection images of *S. aureus* ATCC 29213 cells – a) Untreated cells, b) cells treated with benzyltrimethylammonium bromide, BAB at MIC concentration, c) cells treated for three hours with QOH-C14 at MIC concentration



## ...our previous investigation

## ① aromatic precursor

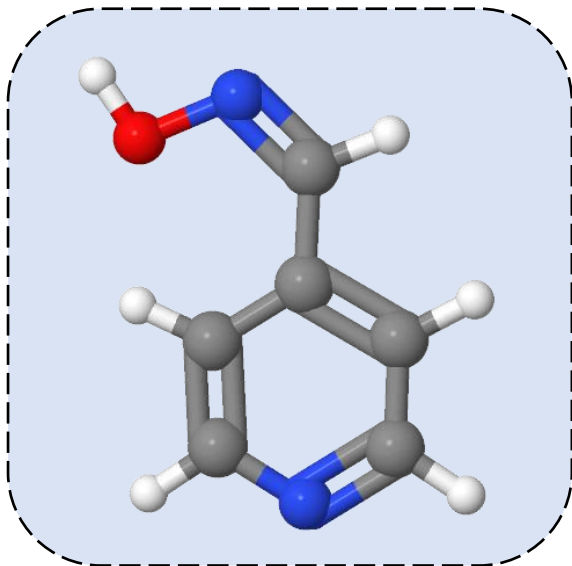


Figure 9. Structure of pyridine-4-aldoxime

## ② long chained terminal bromine atom QACs

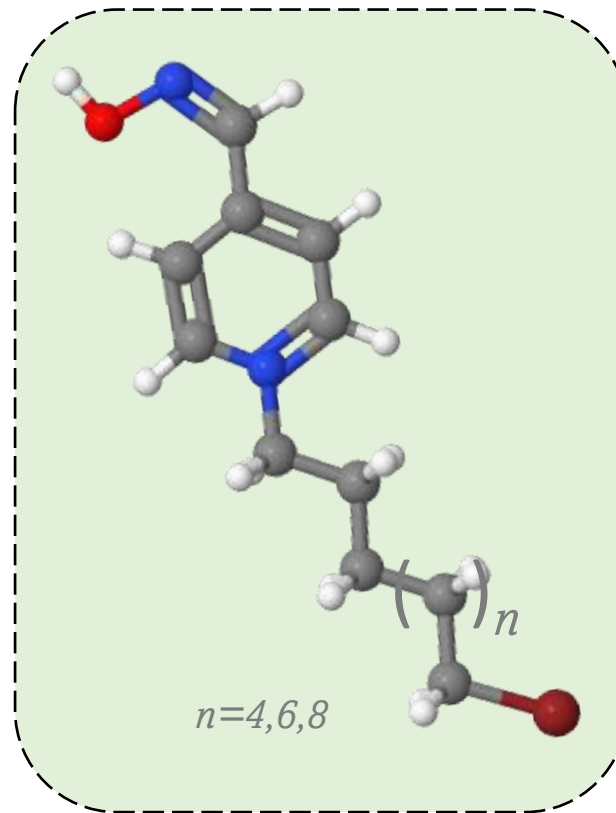


Figure 10. Structure(s) of benzylated pyridine-4-aldoxime QACs

## ③ long chained QAC derivatives

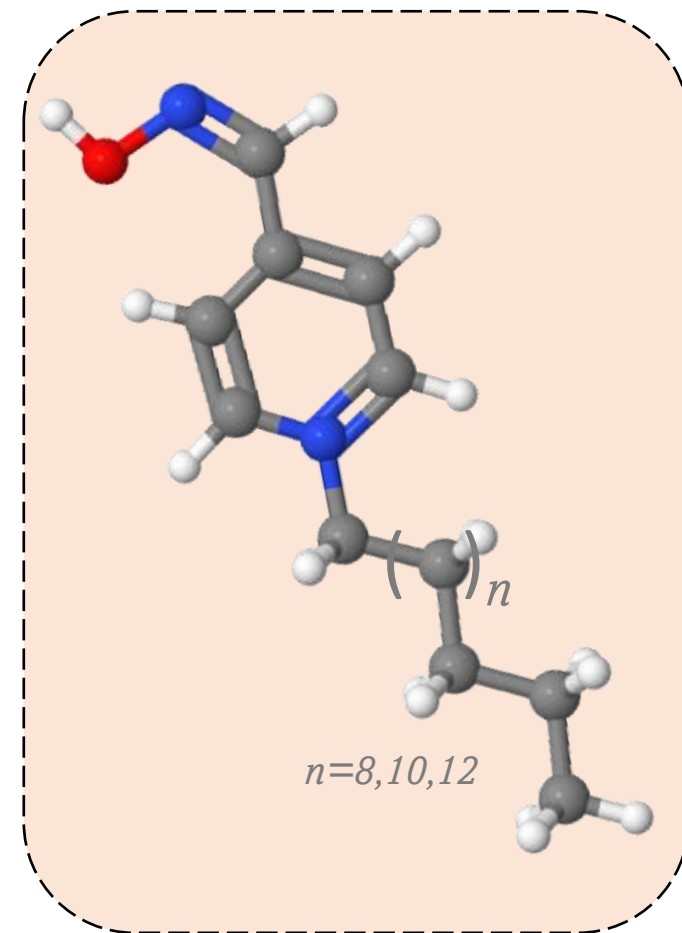
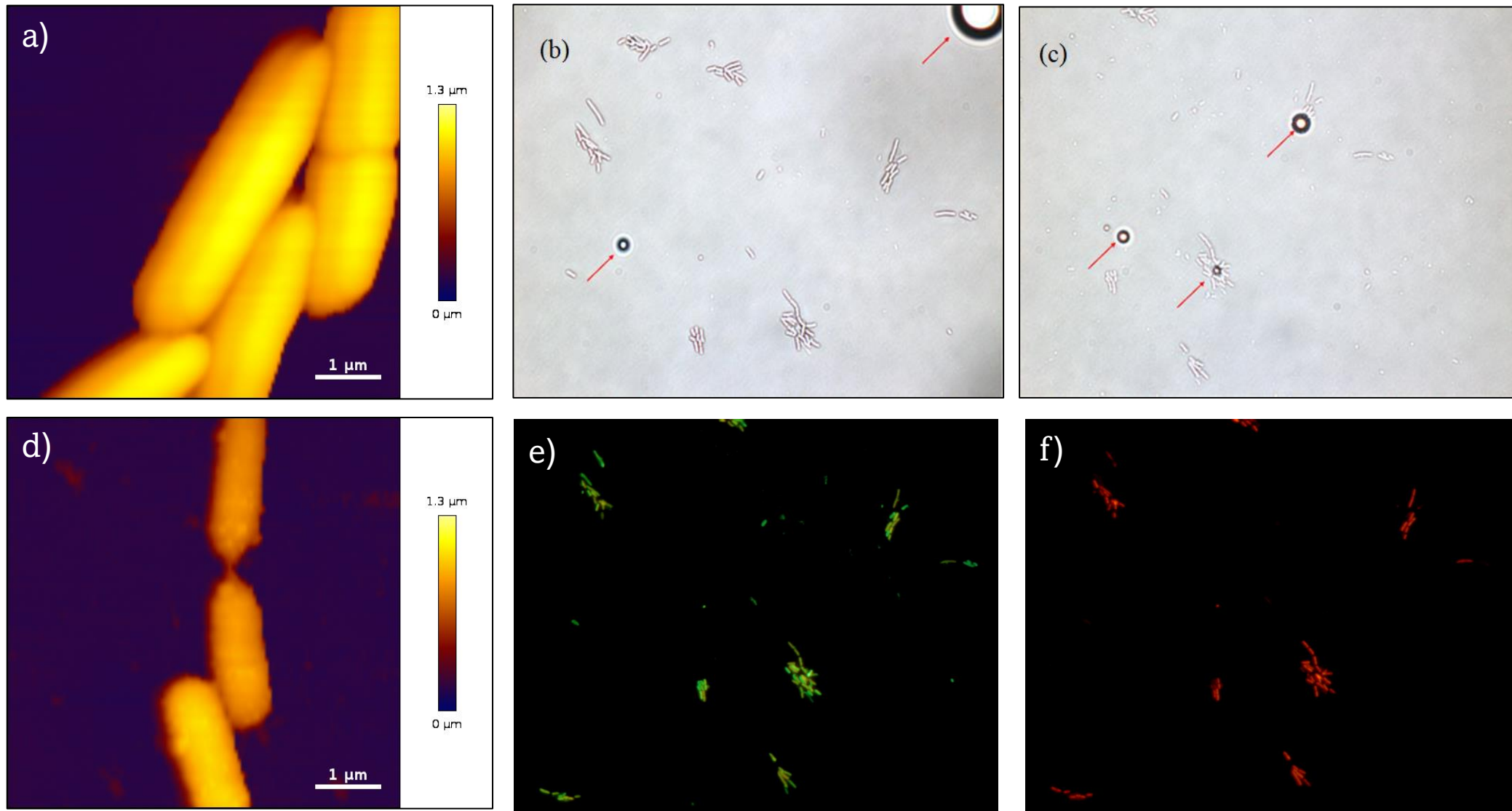


Figure 11. Structure(s) of long chained pyridine-4-aldoxime QACs



**Figure 12.** Atomic force and optical microscopy images of untreated and treated bacterial cells: a) Height AFM image of untreated *Escherichia coli* DH5 $\alpha$ , b) bright-field image of cells at the beginning of treatment, c) bright-field image of the same sample area taken after 3 h of treatment and d) height AFM image of treated bacterial cells, e) SYTO 9 and f) Propidium iodide stained cells upon treatment.

## ...our recent work

1

Synthesis of „soft” QACs

2

Further evaluation of  
biological activity

3

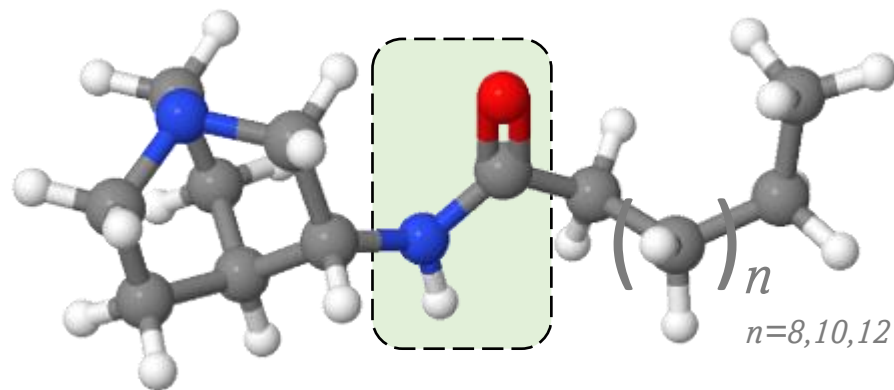
Mode of action  
investigation

Figure 14. General structure(s) of amidoquinuclidine precursor(s)

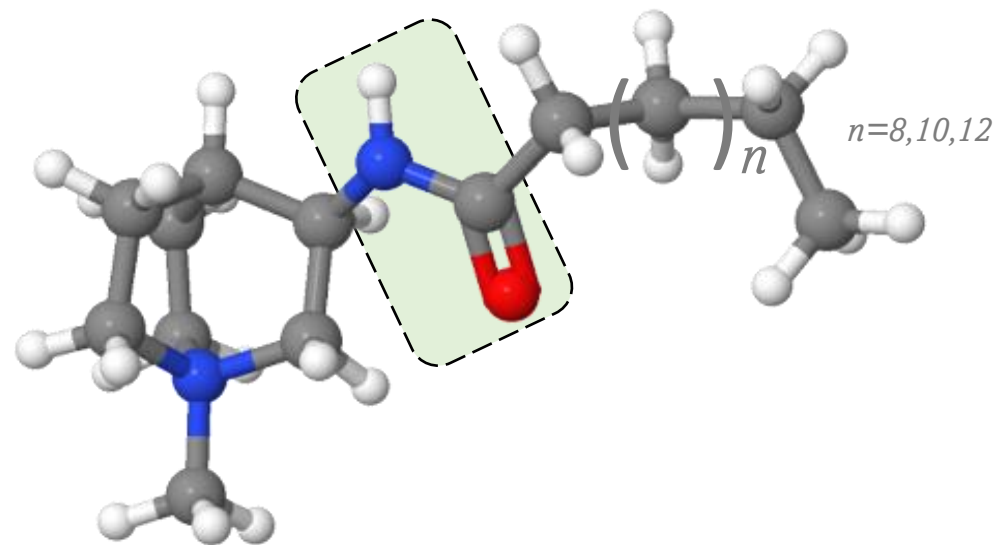


Figure 15. General structure of newly synthesized amide QACs

## ...our recent work

## Membranolytic activity of new 3-amidoquinuclidine QACs

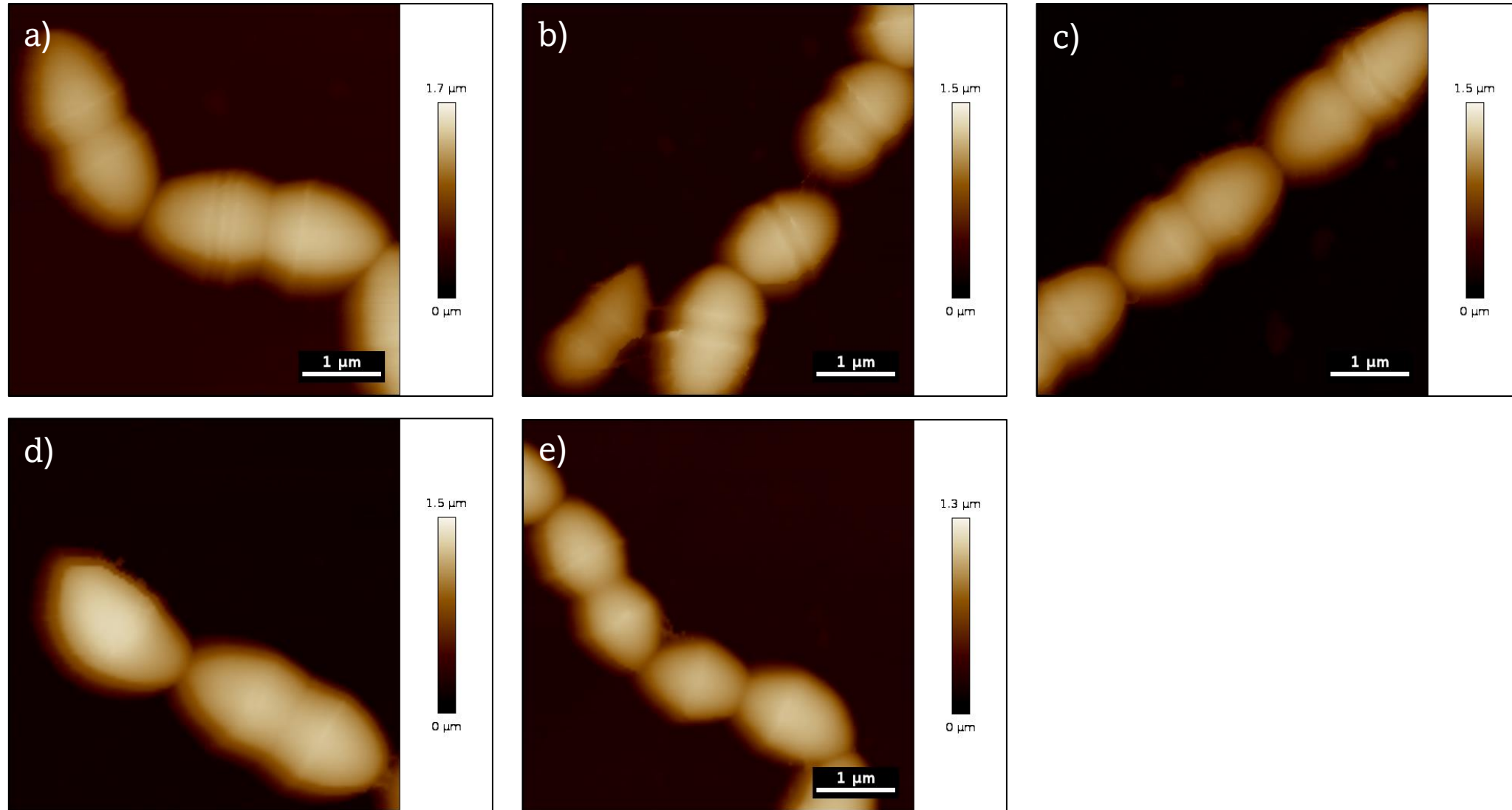
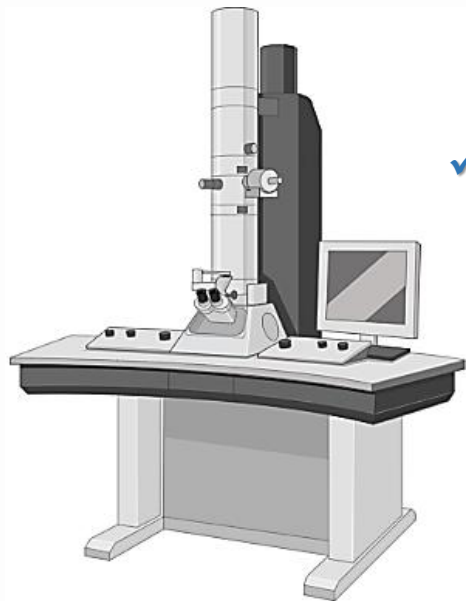


Figure 16. Atomic force microscopy images of a) non treated *Listeria monocytogenes* ATCC 7644, and b) – e) cells treated with 2xMIC concentration of selected most potent quaternary ammonium compounds derived from 3-amidoquinuclidine precursors.



- ✓ imaging of untreated and treated bacteria using scanning electron microscope



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*Thank You!*