

# How Bioactive Glass S53P4 Kills Bacteria

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## Introduction

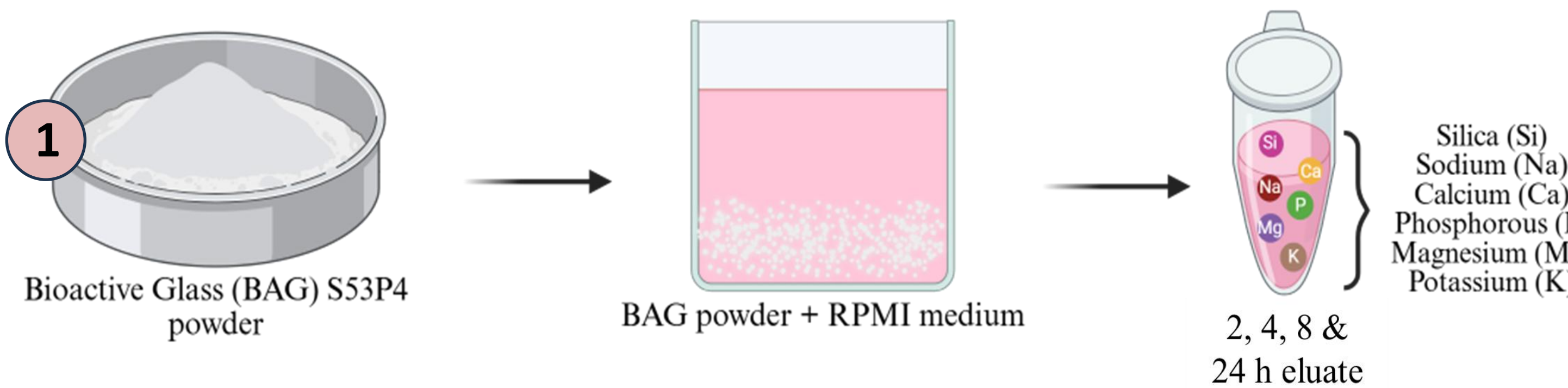
Osteomyelitis remains challenging to treat due to persistent infection and increasing antibiotic resistance. Bioactive glass S53P4 is a clinically used bone substitute with antibacterial and osteogenic properties, but its antibacterial mechanism remains incompletely understood. This study aimed to investigate the cellular and molecular mechanism by which BAG S53P4 kills *Staphylococcus aureus* and *Bacillus subtilis* as model organism.

## Aim

To elucidate the antibacterial mechanism of bioactive glass S53P4 by investigating the cellular, molecular, and ultrastructural changes induced by its ionic dissolution products.

## Methods

### Eluate collection

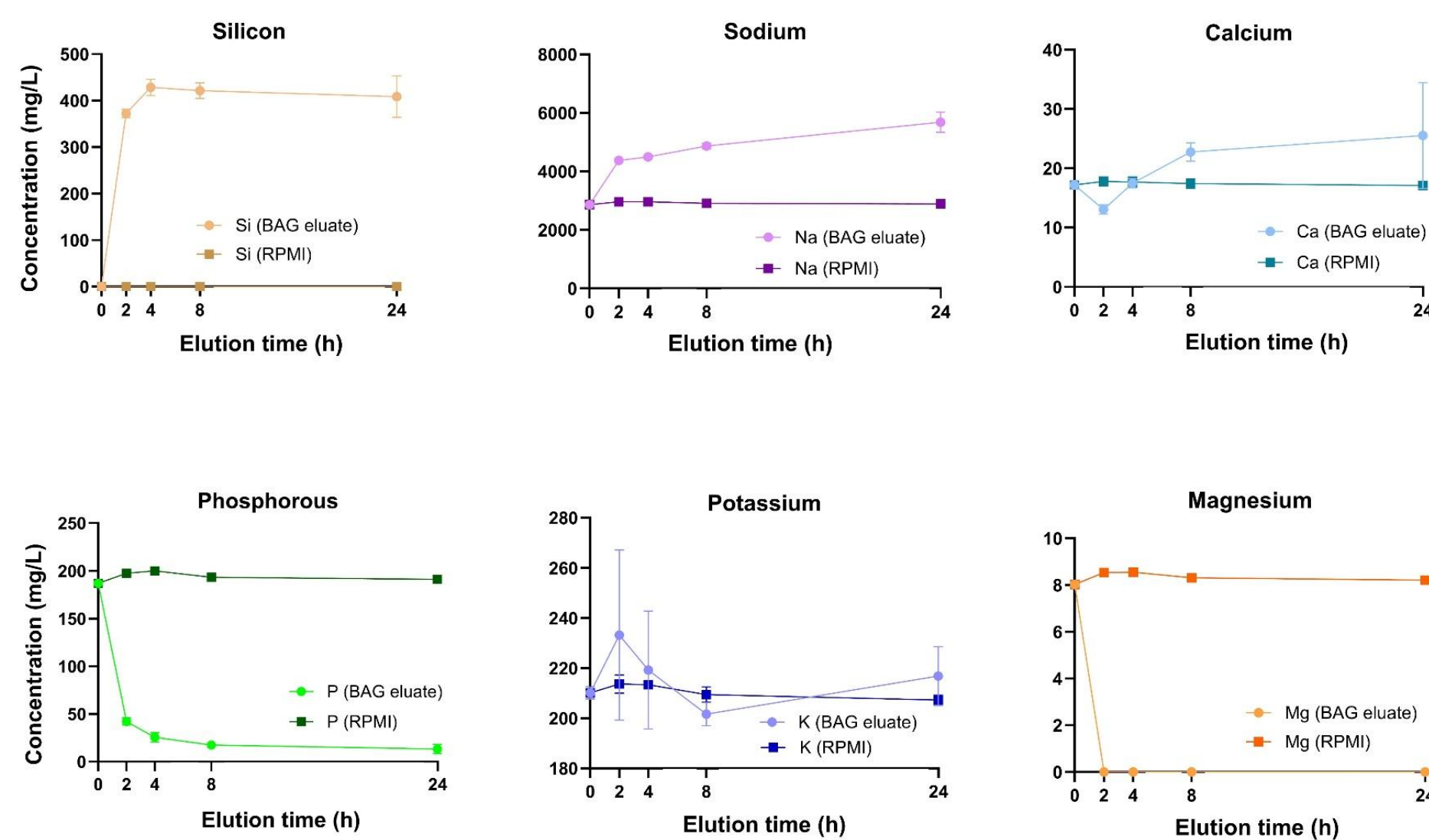


2 Elemental analysis 3 Bactericidal activity 4 SYTOX uptake

5 Fluorescence microscopy 6 RNA-Seq 7 Microscopic analysis (SEM, TEM and EDX)

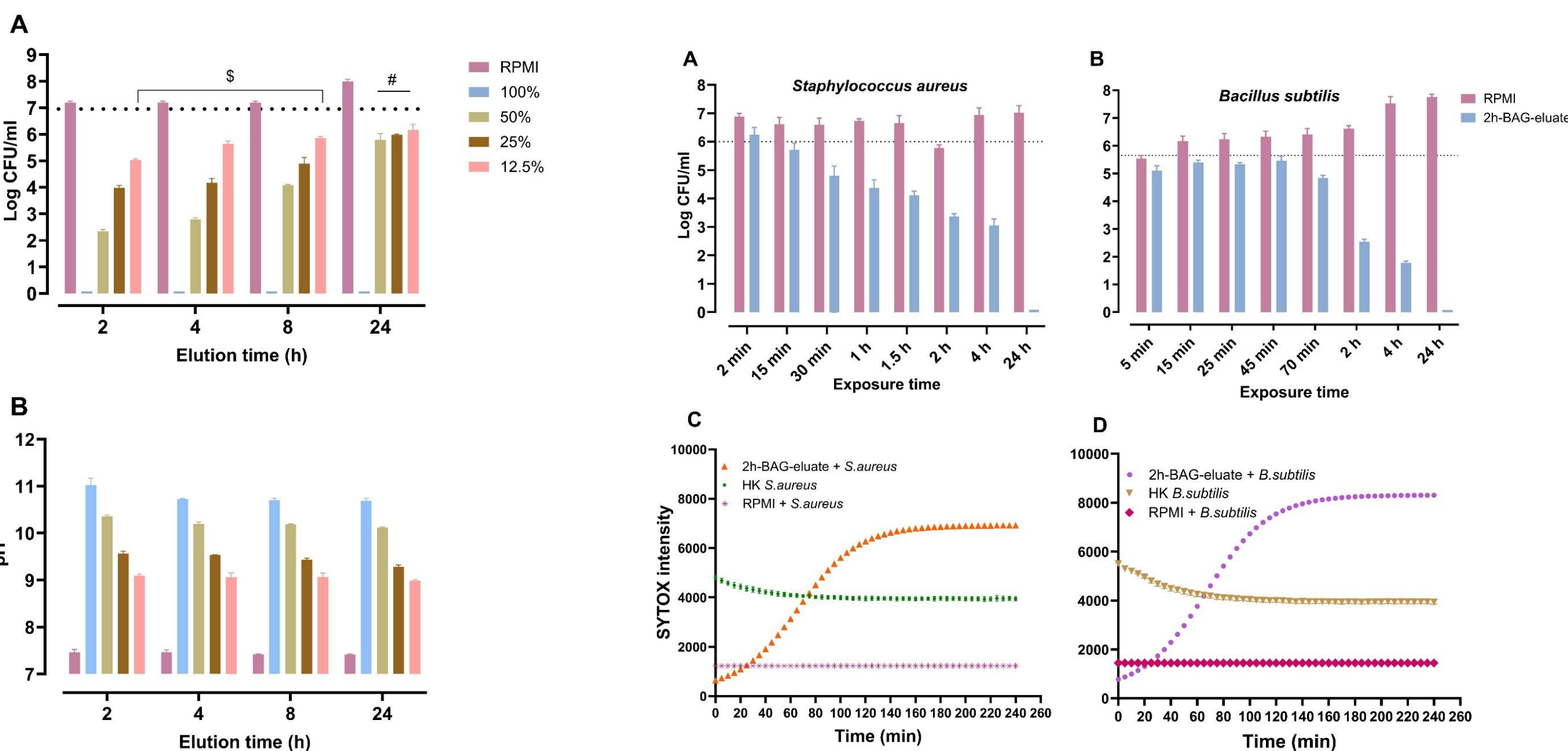
## Results

### Elemental analysis of BAG powder eluates



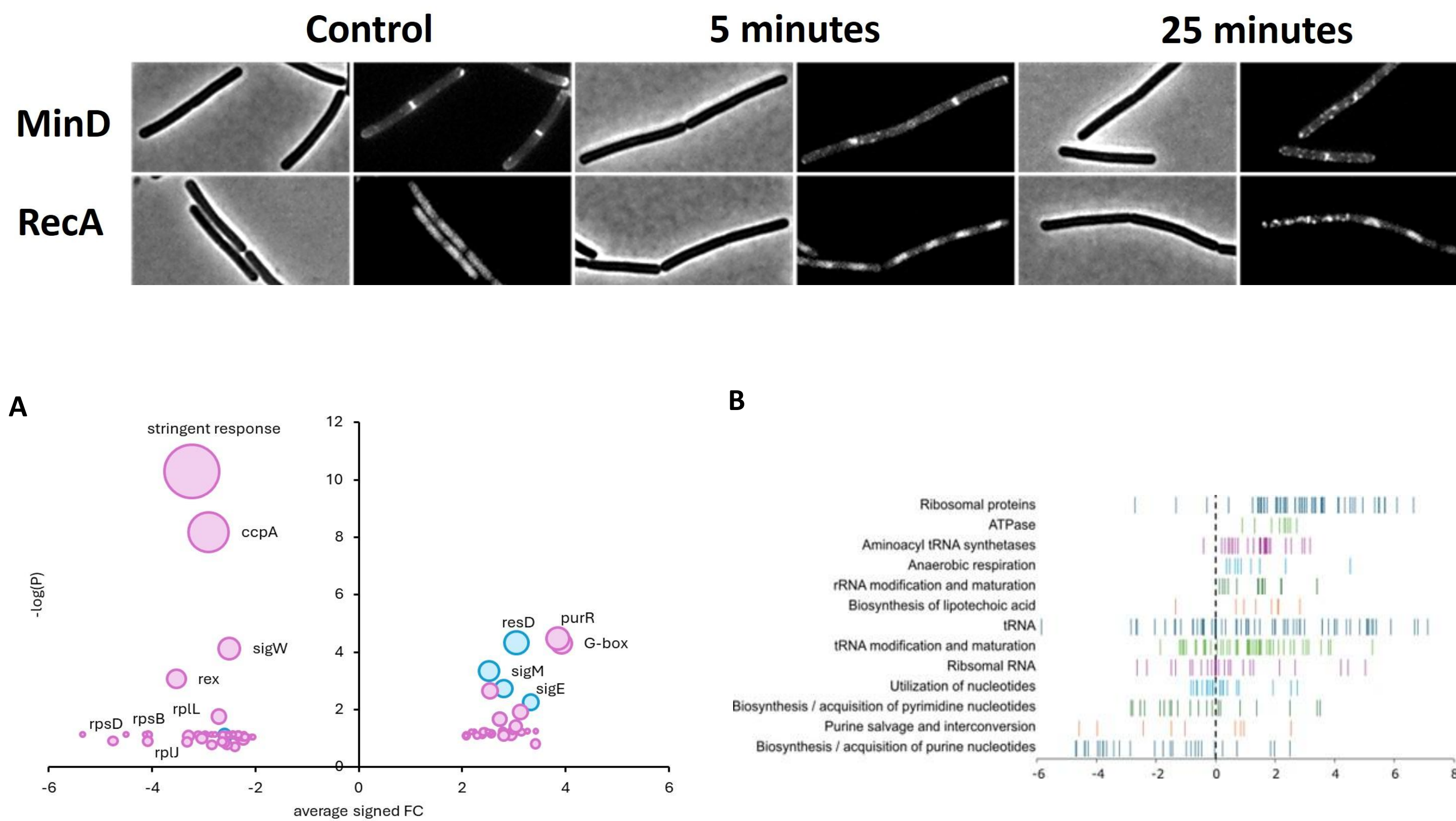
- BAG S53P4 rapidly released Si and Na, followed by Ca, while depleting P and completely removing Mg from the surrounding medium.

### Rapid bactericidal activity of 2h-BAG-eluate by damage to bacterial membrane



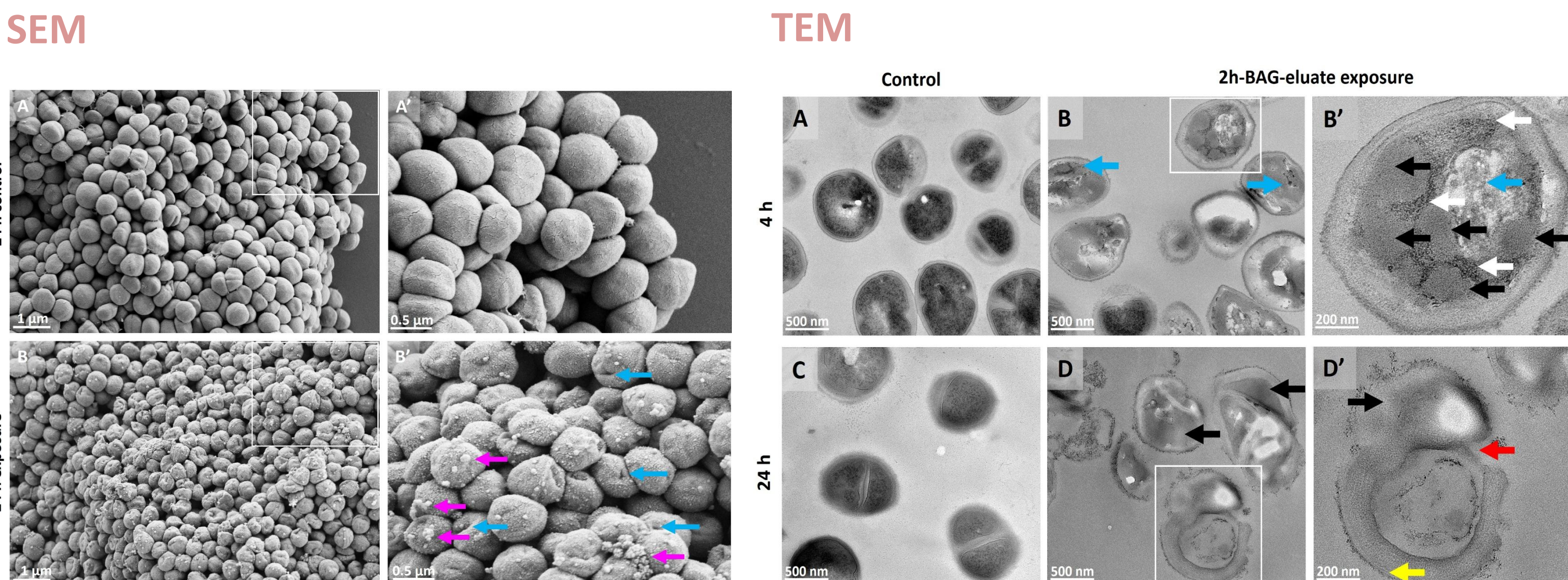
- Early BAG eluates exhibit superior bactericidal activity independent of pH, indicating that factors beyond alkalinity drive bacterial killing.
- Rapid membrane permeabilization coincided with bacterial killing using 2 h eluate

### 2h-BAG eluate rapidly disrupts membrane integrity and induces transcriptomic responses in *B. subtilis*



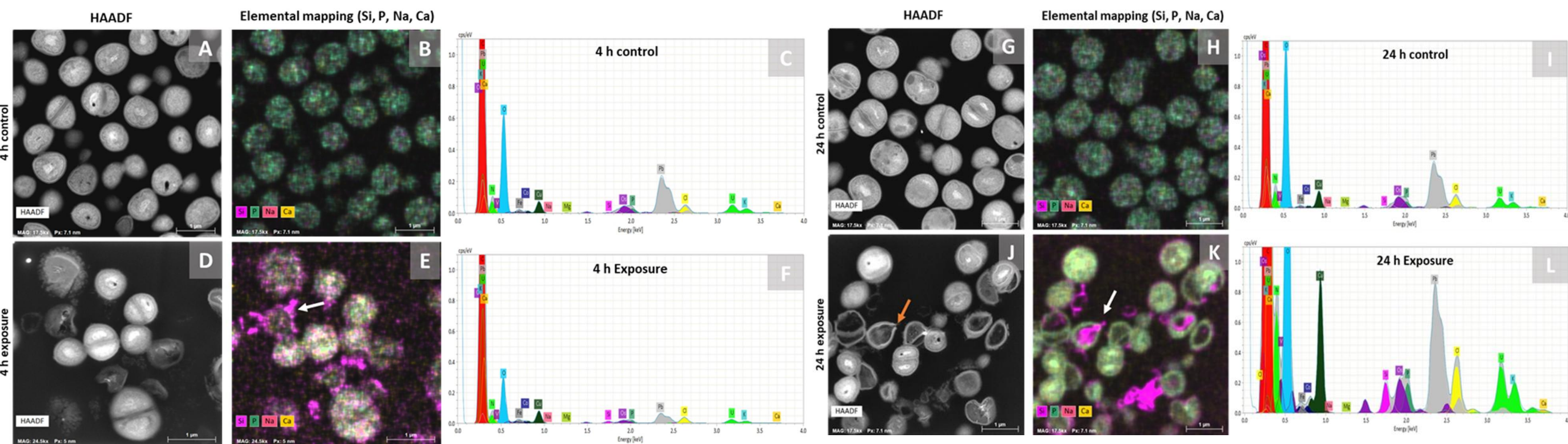
- BAG eluates rapidly disrupted membrane potential and induced nucleoid condensation.
- Early transcriptional remodeling revealed widespread metabolic, energetic and redox stress
- Stress-response regulons were activated, while growth- and biosynthesis-related pathways were repressed.

### Ultrastructural damage of *S. aureus* induced by 2h-BAG-eluate



- BAG eluate exposure caused severe surface damage with particulate deposits on bacterial cells.
- Progressive cell envelope disruption resulted in cytoplasmic leakage and bacterial lysis.

### Time-dependent interaction of BAG-derived ions with *S. aureus* detected by EDX analysis



- Silicon initially deposited on the bacterial surface and later entered cells through cell wall holes formed during structural disruption.

## Conclusions

This study reveals that BAG S53P4 kills bacteria by a dynamic ion-driven mechanism that extends beyond alkaline pH, leading to membrane damage, metabolic dysfunction, cell wall perforation, and bacterial lysis.