

## Supplementary Information for

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Combined electric field treatment with copper and silver for water  
disinfection

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### 1. Supplementary Text

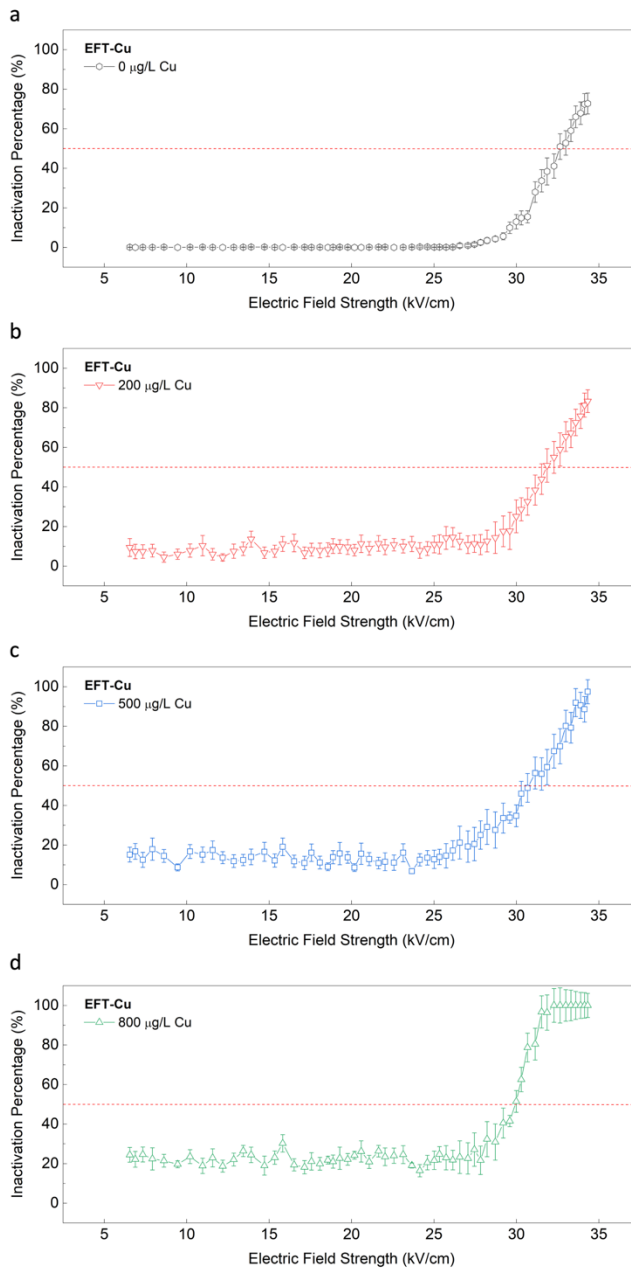
Supplementary Text 1. Ag ion inactivation mechanisms

Regarding the mechanism in which Ag ions inactivate bacteria more specifically, due to the affinity of Ag ions towards thiol groups in the membrane structure, membrane proteins containing sulfhydryl groups will typically act as the binding sites, causing gradual degradation of the proteins and inactivation of the cell. (Xu et al., 2021)

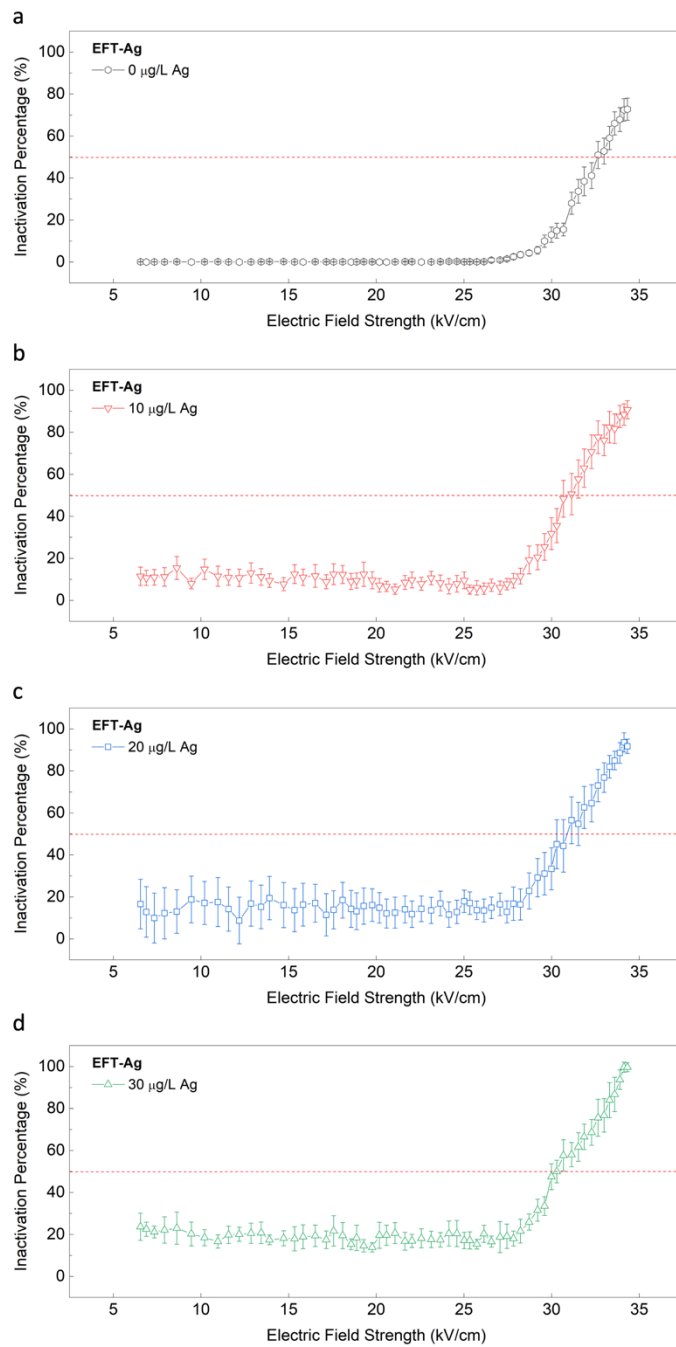
Studied by Jung et al., *S. aureus* and *E. coli* exposed to Ag ions demonstrated damage to the biomolecules responsible for maintaining division and growth of the bacteria, afterwards, the plasma membrane of the bacteria would shrink or fall off the walls, leading to inactivation. (Jung et al., 2008) In another study, Ag ions showed destruction of the dehydrogenase in the respiratory chain, inhibiting respiration and interfering with growth and metabolism in bacteria. (Li et al., 2010a) Additionally,

Holt and Bard discovered that Ag could bind to the enzymes in the transport and respiratory chains, which could then lead to inhibited enzyme activity, imbalance in the transmembrane proton pump, and irreversible damage to the respiratory chain due to the instability of the proton gradient both inside and outside the membrane. (Holt and Bard, 2005) This uncontrollable respiration led to the production of excessive ROS, in which the Ag ions played an auxiliary catalytic role.

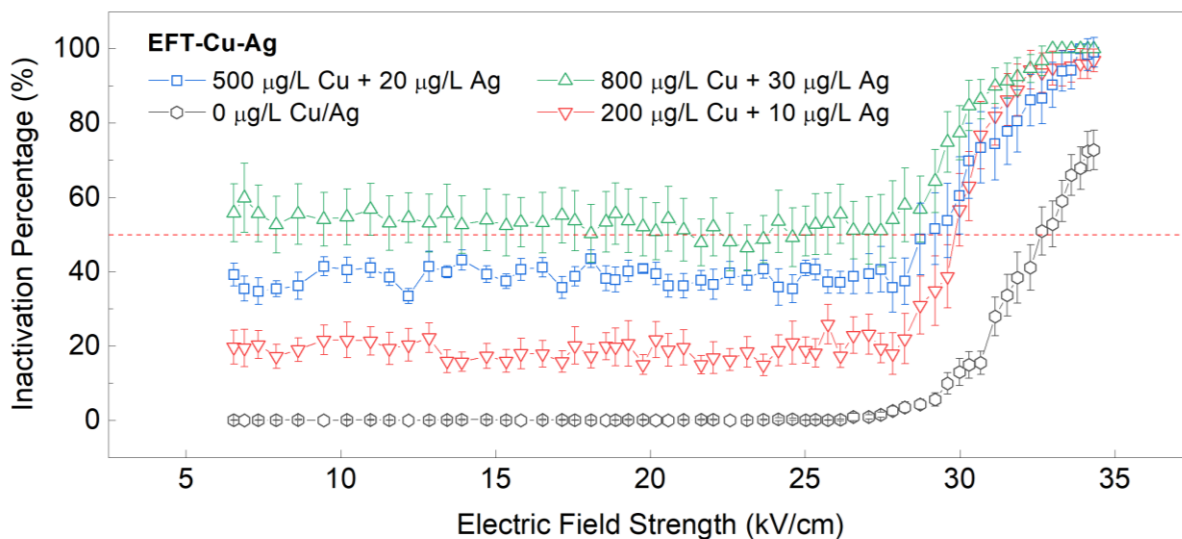
## 2. Supplementary Figures



**Fig. S1 | Inactivation percentage for EFT-Cu concentrations 0-800 µg/L.** Error bars represent 95% confidence intervals from the mean values for all analyzed replicates out of a total 30 repeat channels for each individual experiment for Cu concentrations 0 µg/L (a), 200 µg/L (b), 500 µg/L (c), and 800 µg/L (d), respectively. The red dashed line indicated the lethal electroporation threshold where 50% inactivation is achieved.



**Fig. S2 | Inactivation percentage for EFT-Ag concentrations 0-30 µg/L.** Error bars represent 95% confidence intervals from the mean values for all analyzed replicates out of a total 30 repeat channels for each individual experiment for Ag concentrations 0 µg/L (a), 10 µg/L (b), 20 µg/L (c), and 30 µg/L (d), respectively. The red dashed line indicated the lethal electroporation threshold where 50% inactivation is achieved.



**Fig. S3 | Inactivation percentage for EFT-Cu-Ag for varying concentrations.** Error bars represent 95% confidence intervals from the mean values for all analyzed replicates out of a total 30 repeat channels for each individual experiment. The red dashed line indicated the lethal electroporation threshold where 50% inactivation is achieved.

### SI References

Holt K B, Bard A J (2005). Interaction of Silver(I) Ions with the Respiratory Chain of *Escherichia coli*: An Electrochemical and Scanning Electrochemical Microscopy Study of the Antimicrobial Mechanism of Micromolar  $\text{Ag}^+$ . *Biochemistry*, 44(39): 13214-13223

Jung W K, Koo H C, Kim K W, Shin S, Kim S H, Park Y H (2008). Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. *Applied and environmental microbiology*, 74(7): 2171-2178

Li W-R, Xie X-B, Shi Q-S, Zeng H-Y, Ou-Yang Y-S, Chen Y-B (2010a). Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. *Applied Microbiology and Biotechnology*, 85(4): 1115-1122

Xu Z, Zhang C, Wang X, Liu D (2021). Release Strategies of Silver Ions from Materials for Bacterial Killing. *ACS Applied Bio Materials*, 4(5): 3985-3999