Supporting Information

Near-atomic Three-Dimensional Mapping for Site Specific Chemistry of 'Superbugs'

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FIB lift-out preparation for APT specimen retrieved from bacterial cells

The FIB lift-out procedure for preparation of APT specimens from bacterial cells is based on established protocols[1, 2] and summarized in Supporting Figure 1. A dual-beam FIB-SEM instrument (Quanta 3D FEG, FEI Company) equipped with a chamber-installed micromanipulator (MM3A-EM, Kleindiek Nanotechnik GmbH) was utilized. The detailed steps of the approach are summarized in three phases:

Phase 1: Wedge preparation (Supporting Figure 1a-c)

Using a low-current voltage electron beam, the substrate was rapidly scanned for a target single bacterium (indicated by the arrow in Supporting Figure 1a-b). After locating an individual cell, a rectangular Pt protective layer of thickness of 1.5-2 μm was deposited on top of the bacterium. Using the Ga^+ ion beam, trenches were also milled around three sides of the coated bacterium to facilitate the creation of a wedge from the target cell (Supporting Figure 1c).

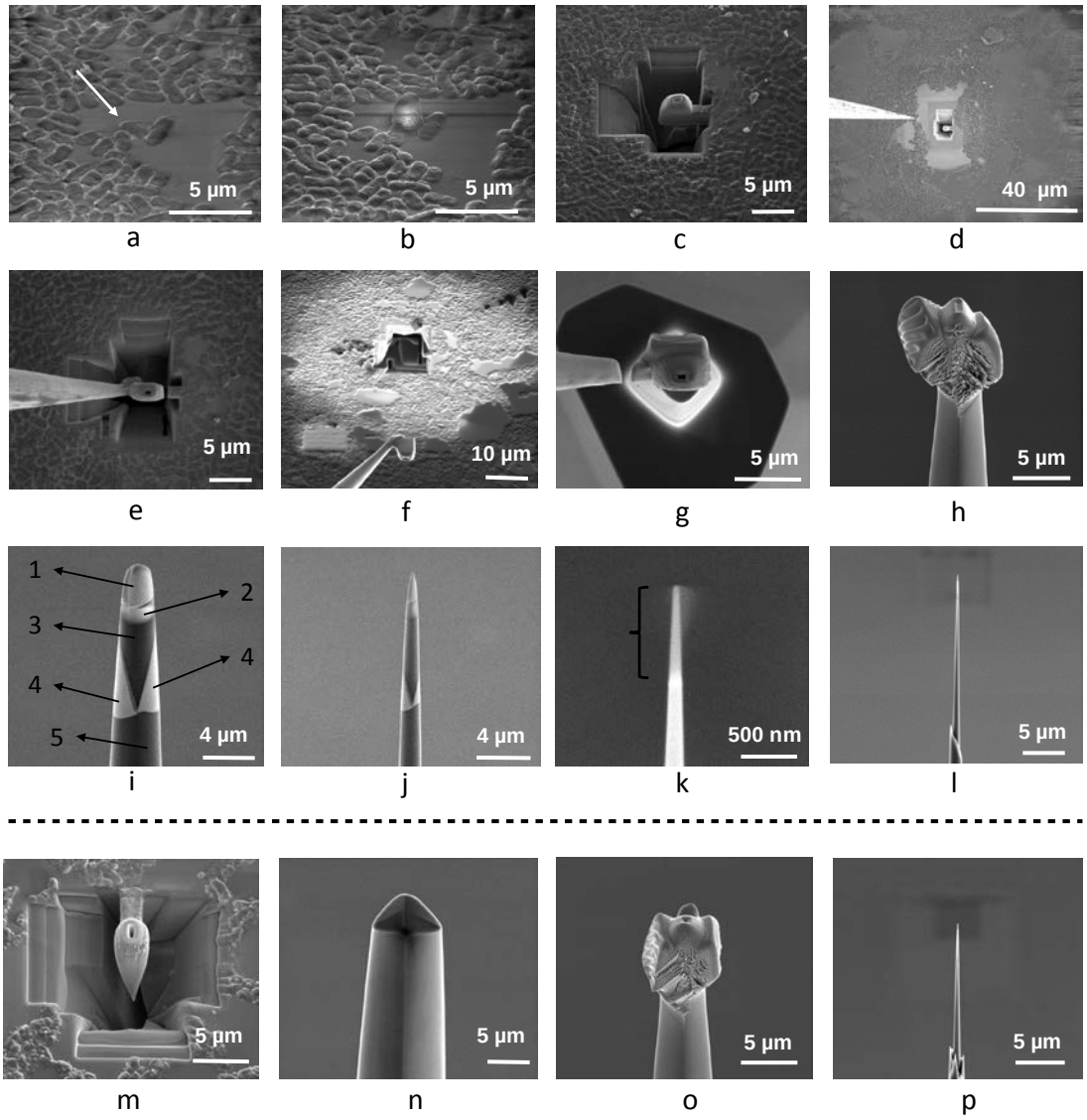
Phase 2: Wedge Lift-out (Supporting Figure 1d-h)

As shown in Supporting Figure 1d-e, the micromanipulator needle approached the specimen and was subsequently attached to the bacterial cell wedge using Pt deposition. After milling underneath from an angle and then also the fourth attached side, the wedge of bacterial cell became free from the substrate (Supporting Figure 1e) and was simply lifted out (Supporting Figure 1f) by dropping the microscope stage. Next, the bacterial cell lift-out wedge was attached to a Si support post, again with Pt deposition, and cut free from the micromanipulator needle by Ga^+ ion milling with a cleaning cross section (Supporting Figure 1g-h).

Phase 3: Annular milling (Supporting Figure 1i-l)

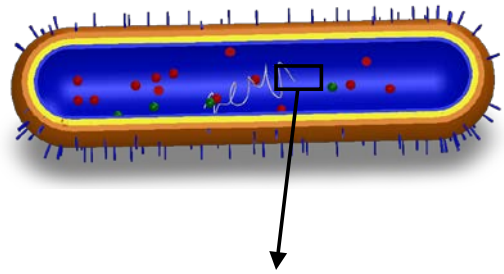
In this final phase, the desired needle form for the APT specimen was fabricated by FIB milling using a series of annular patterns, where the inner and outer diameters were gradually decreased as the ion voltage/current values were reduced to sharpen the specimen (Supporting Figure 1i-l). Several regions were labelled on Supporting Figure 1i, where region 1 corresponds to the Pt protective layer, region 2 contains the material of interest from the bacterial cell, and the Si substrate (upon which the bacterial cells were prepared) and Pt attachment are shown as regions 3 and 4, respectively. Region 5 indicates the Si support post upon which the lift-out material was attached. In Supporting Figure 1k, the Pt protective layer was completely removed and the bacterial cell portion was marked at the very end of the tip. The end tip diameter is less than 60 nm. Finally, needle-shaped specimens were checked for any secondary tips created during annular milling and cut/milled away as necessary to ensure a distance of ~10 μm between the end tip and any secondary tips lower down the shank of the needle.

During the APT experiment, tip specimens experience field induced mechanical stresses in the order of 10^9 to 10^{10} Pa [3-5]. Therefore, it is critical to maintain a strong attachment between the lift-out wedge and the support post to avoid tip failure. In phase 1 of the tip specimen preparation, a V-shape geometry was located at the bottom of the lift-out wedge (see side view of the wedge in Supporting Figure 1m), and in the current study, an inverted V-shape geometry was also milled on top of the support post, as shown in Supporting Figure 1n. Subsequently, these V-shaped geometries were carefully aligned, and the gaps between them were filled with Pt deposition (Supporting Figure 1o). It is noted that this process of creating freshly cut/milled surfaces on the support post also has the benefit of removing oxide layers that could weaken the attachment after Pt deposition. The final APT specimens were checked for any secondary points below the end tip (Supporting Figure 1p).

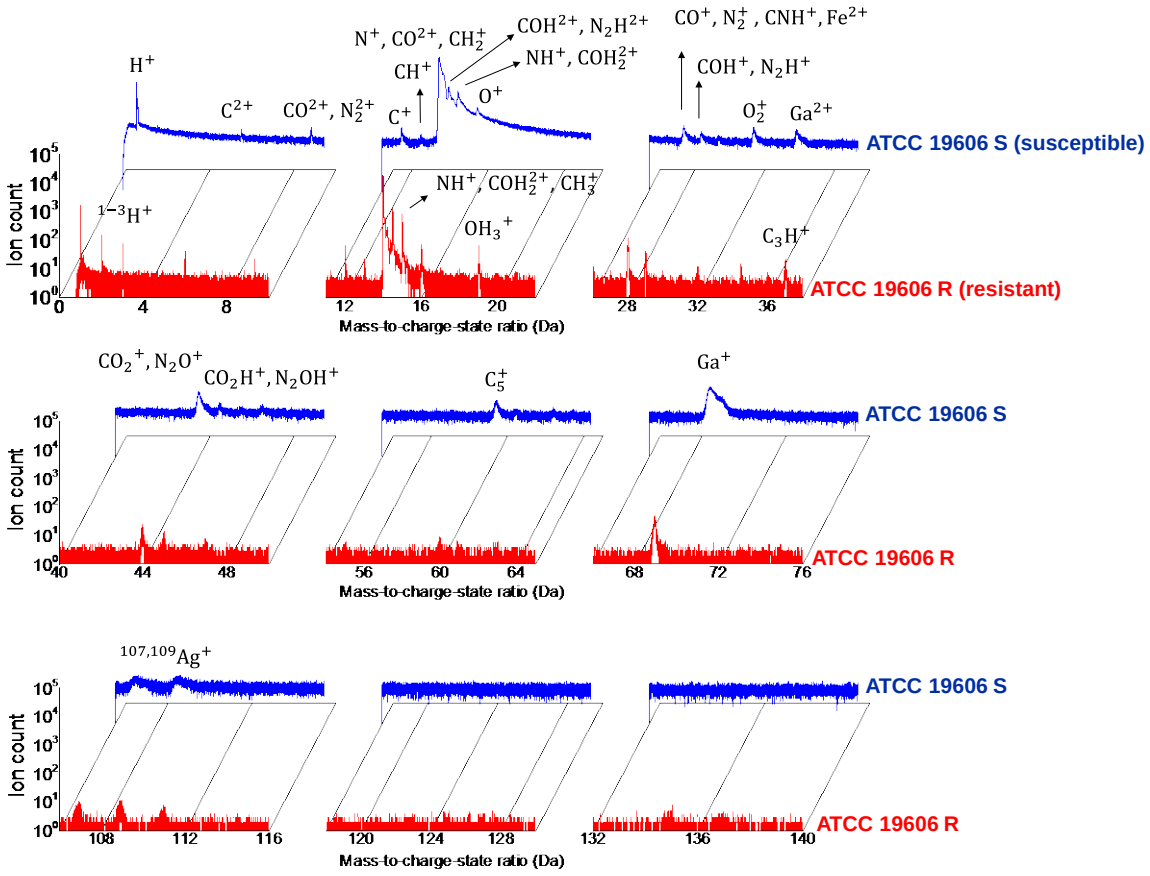


Supporting Figure 1: SEM images taken during the FIB lift-out process to create APT specimen from bacterial cells.

Mass-to-charge-state spectra acquired from intracellular domain of susceptible and resistant strains of A. baumannii.



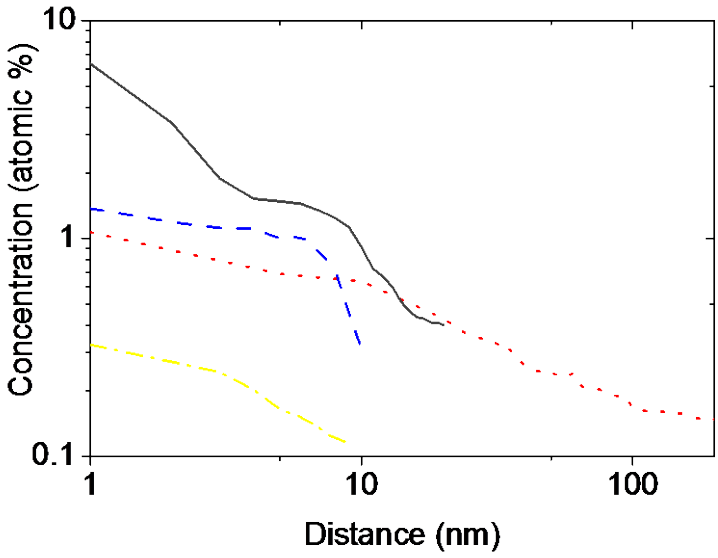
Mass spectra from intracellular domain of susceptible and resistant strains



Supporting Figure 2: Mass-to-charge-state ratio spectra acquired from the intracellular domain of *A. baumannii*, revealing distinct information from both a susceptible strain (ATCC 19606 S) highlighted in blue, and a resistant strain (ATCC 19606 R) highlighted in red.

1D atomic concentration (%) profile of Ag along the z-axis of the tomographic reconstruction

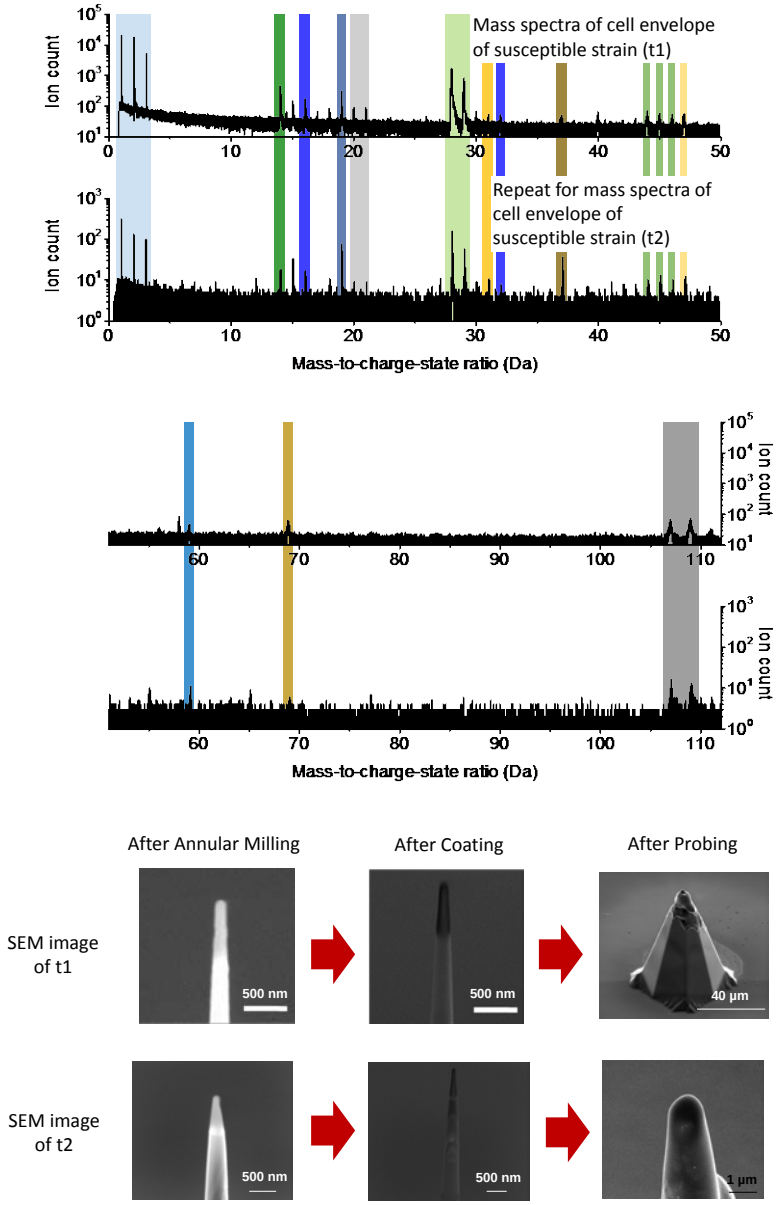
Supporting Figure 3 shows 1D atomic concentration (%) profile of Ag along the z-axis of the tomographic reconstructions of the specimen needle from the cell envelope of susceptible strain (ATCC 19606 S) – dark grey curve (straight line), cell envelope of resistant strain (ATCC 19606 R) – blue curve (dashed line), intracellular domain of susceptible strain (ATCC 19606 S) – red curve (dotted line), and intracellular domain of resistant strain (ATCC 19606 R) – yellow curve (dashed dotted line).



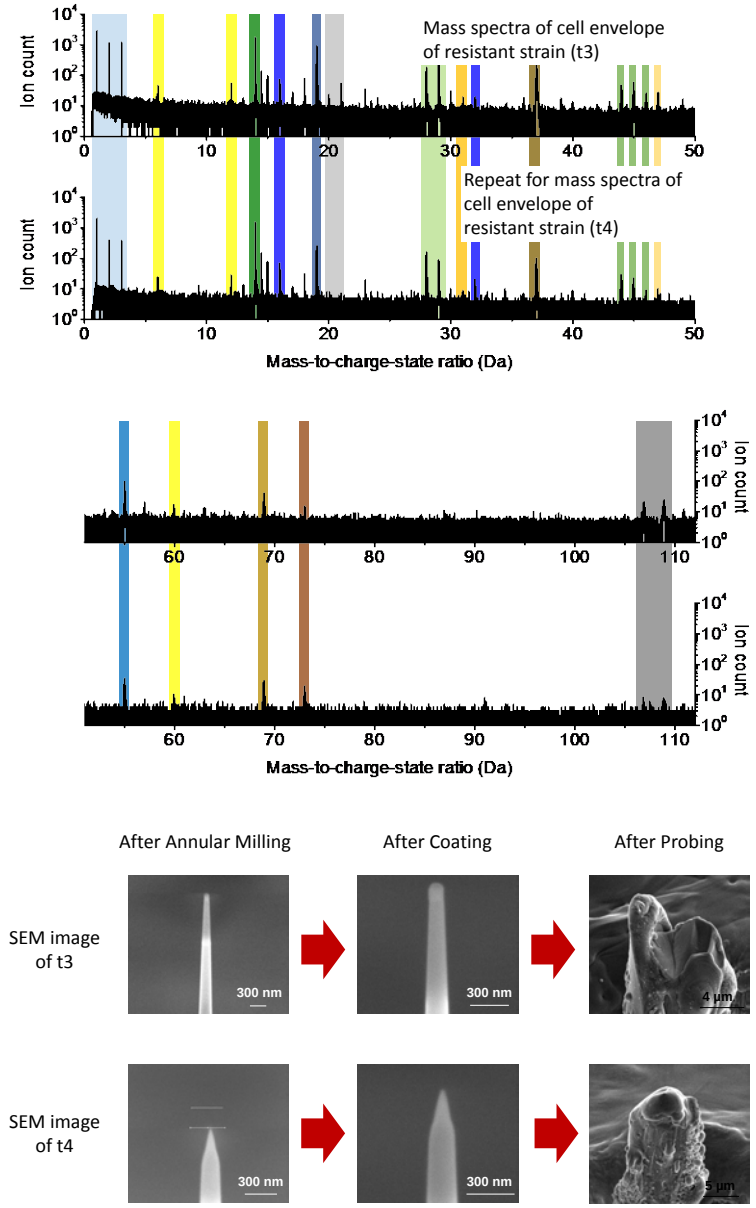
Supporting Figure 3: 1D atomic concentration (%) profile of Ag along the z-axis of the tomographic reconstruction. Cell envelope of susceptible strain (ATCC 19606 S) – dark grey curve (straight line), cell envelope of resistant strain (ATCC 19606 R) – blue curve (dashed line), intracellular domain of susceptible strain (ATCC 19606 S) – red curve (dotted line), and intracellular domain of resistant strain (ATCC 19606 R) – yellow curve (dashed dotted line).

Repeats of experiments

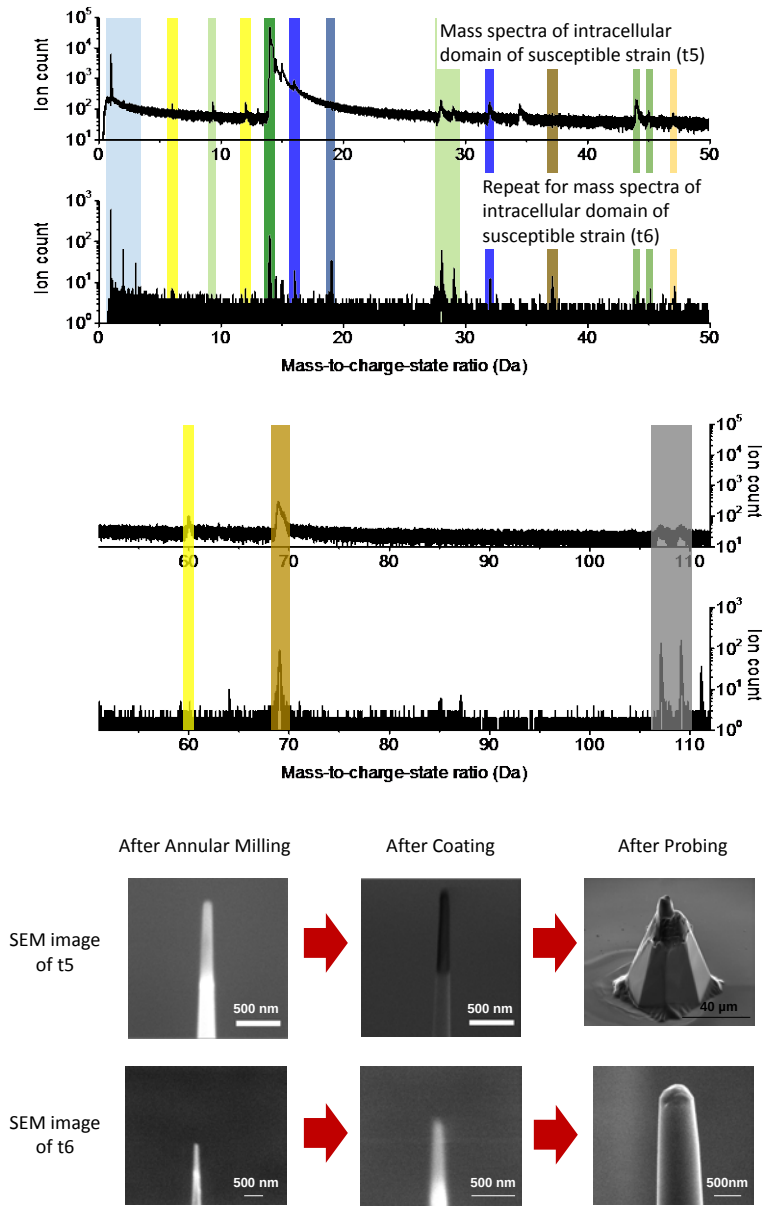
Supporting Figures 4-7 represent mass-to-charge-state spectra for the original data and one repeat acquired from the cell envelope of susceptible strain (ATCC 19606 S), cell envelope of resistant strain (ATCC 19606 R), intracellular domain of susceptible strain (ATCC 19606 S), and intracellular domain of resistant strain (ATCC 19606 R) respectively, along with corresponding SEM images of the tips before coating (in fact after tip sharpening), after nanoscale Ag coating, and after probing.



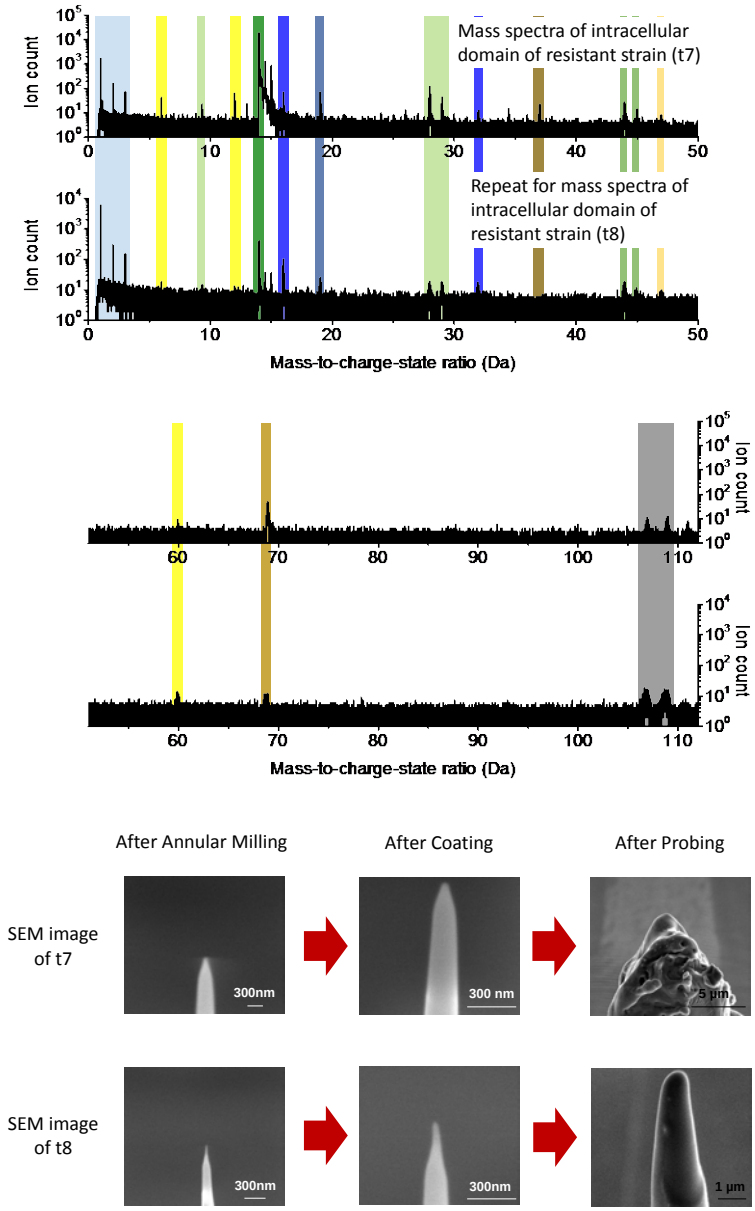
Supporting Figure 4: Mass-to-charge-state spectra for the original data and one repeat collected from cell envelope of susceptible strain (ATCC 19606 S). Also, the SEM images of the corresponding tips after sharpening, after coating and after probing are presented. Note that t1 stands for tip 1, and t2 stands for tip 2.



Supporting Figure 5: Mass-to-charge-state spectra for the original data and one repeat collected from cell envelope of resistant strain ((ATCC 19606 R)). Also, the SEM images of the corresponding tips after sharpening, after coating and after probing are presented. Note that t3 stands for tip 3, and t4 stands for tip 4.



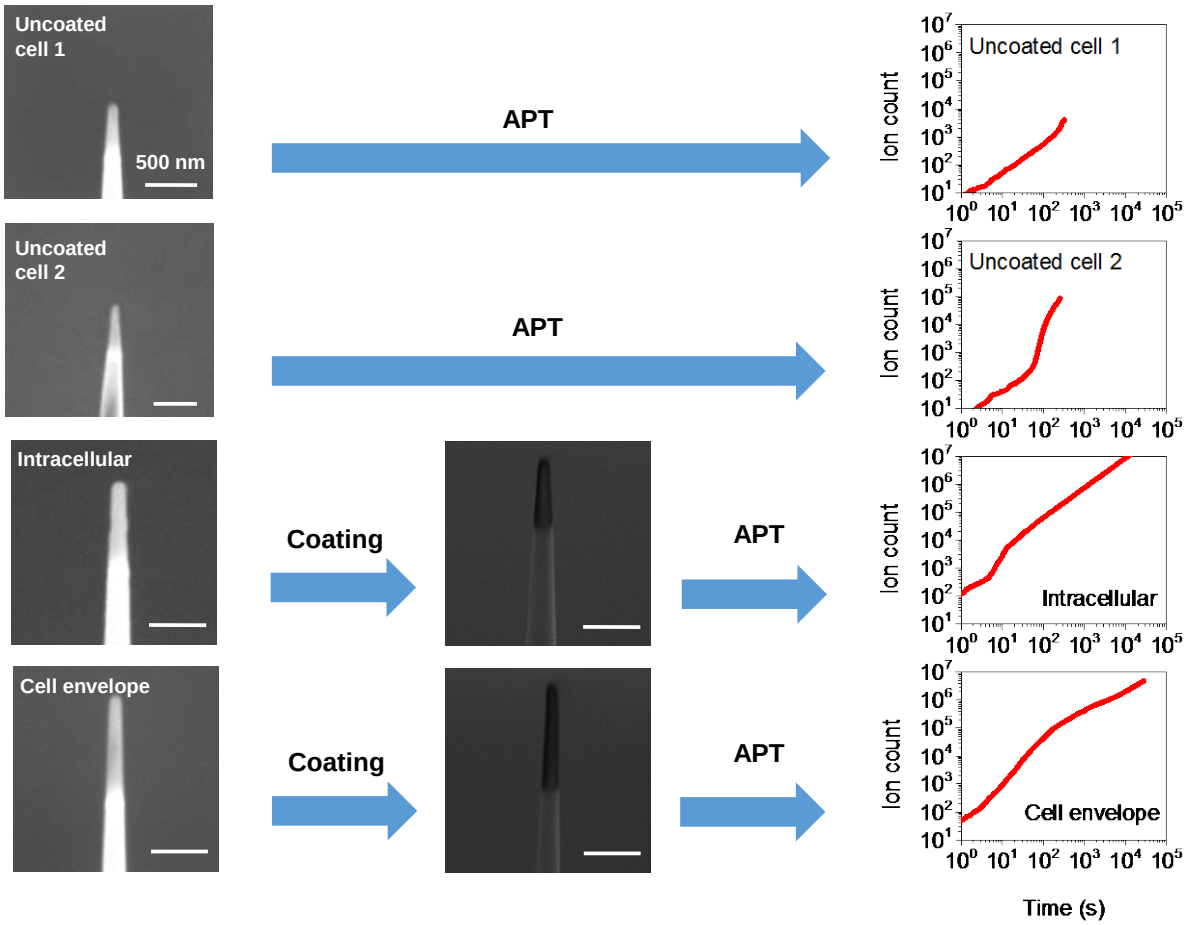
Supporting Figure 6: Mass-to-charge-state spectra for the original data and one repeat collected from intracellular domain of susceptible strain (ATCC 19606 S). Also, the SEM images of the corresponding tips after sharpening, after coating and after probing are presented. Note that t5 stands for tip 5, and t6 stands for tip 6.



Supporting Figure 7: Mass-to-charge-state spectra for the original data and one repeat collected from intracellular domain of resistant strain ((ATCC 19606 R)). Also, the SEM images of the corresponding tips after sharpening, after coating and after probing are presented. Note that t7 stands for tip 7, and t8 stands for tip 8.

Performance of Metallic Coating for Pulsed-Voltage APT of Bacterial Cells

The Ag-coated bacterial cell APT specimens, together with the uncoated control specimens, were subsequently subjected to pulsed-voltage atom probe experiments, and the number of ions collected over time was recorded and presented in Supporting Figure 8 to compare the field evaporation behavior. It is evident that the APT experiments of the coated specimens were much more successful than the uncoated controls, with two orders of magnitude difference in the time and number of ions collected (note the log scales in the Supporting Figure 8).



Supporting Figure 8: Plots of ion counts over time indicate the field evaporation behavior for the representative un-coated and coated APT specimens that were probed under pulsed-voltage condition.

Supporting Information References

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