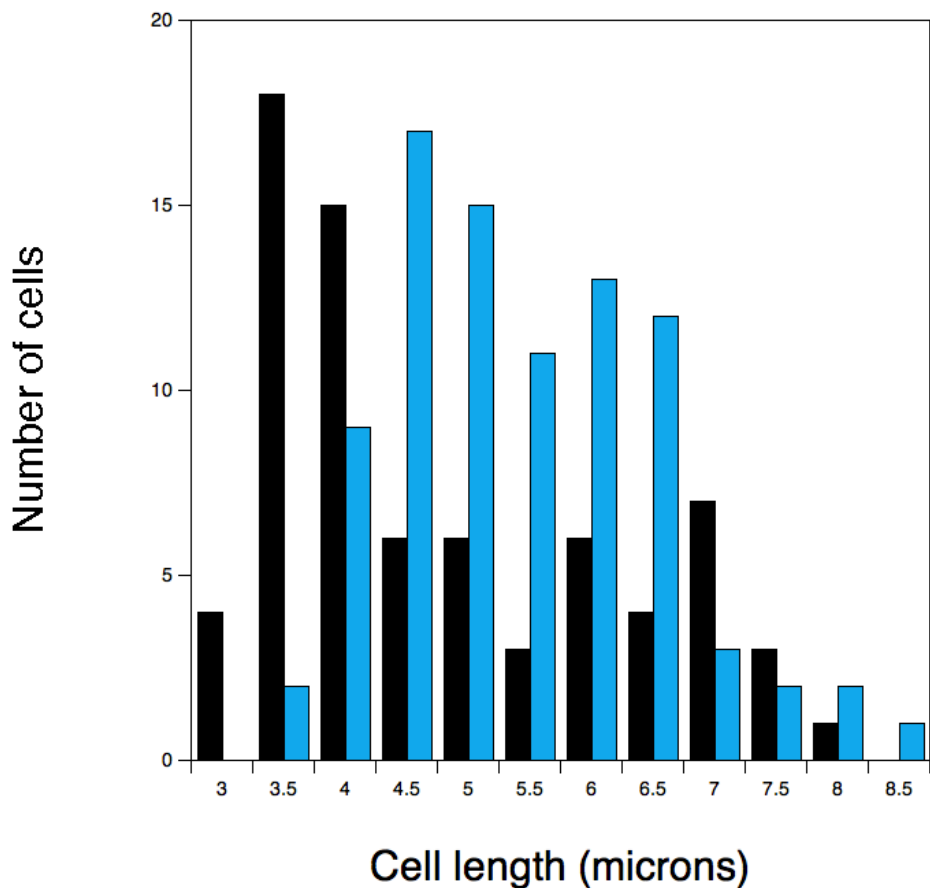


## Supplemental Material

### Does daptomycin inhibit cell division?

The ability of daptomycin to intensely label septa raised the question: does daptomycin inhibit cell division? If it did, we would expect cells exposed to daptomycin to be significantly longer than untreated cells. To address this question, we grew cells on an agarose pad in either the presence or absence of daptomycin for 1 hour at 30°C and then measured their cell lengths. The cell length distribution of the population (Figure S1) for the daptomycin treated cells (blue bars) is only slightly shifted (longer) compared to the untreated population (black bars) and the average cell length for cells grown in the presence of daptomycin (5.7µm, n=87) was only slightly longer compared to the control (5.0µm, n=73). It would appear that even though daptomycin intensely binds to septa, it does not specifically inhibit cell division.



**Figure S1.** Cells grown in the presence of daptomycin are only slightly longer than untreated cells. *B. subtilis* (AD3007) cells were grown on an agarose pad either with 10 µg/ml daptomycin (blue bars) or without daptomycin (black bars) for 1 hour. Cell lengths were measured and the values grouped into bin sizes of 0.5 micron. The

histogram shows the number of cells belonging to each size bin plotted versus cell length.

### **Supplemental movies.**

**Movie S1.** A time-lapse series showing a field of *B. subtilis* cells grown in the presence of a lethal dose of daptomycin (10 $\mu$ g/ml) on an agarose pad at 30°C. Time points are five minutes apart. The sequence begins after 25 minutes of daptomycin exposure. The vital stain FM 4-64 shows that by this time point, many of the cells have large membrane foci or protrusions, a sign of extensive membrane damage. The stain sytox green is excluded from cells with intact membranes but brightly stains dead cells. Over time, nearly all of the cells stain brightly with sytox green. Upon death, cells decrease slightly in size due to loss of turgor pressure. Scale bar equals 5 microns.

**Movie S2.** A time-lapse series showing a field of *B. subtilis* cells grown in the presence of the vital stains FM 4-64 and sytox green on an agarose pad at 30°C. Time points are five minutes apart. The fluorescence from the membrane stain FM 4-64 is uniform throughout the cell and fluorescence from stain sytox green is faint due to exclusion from cells with intact membranes.

**Movie S3.** A small field of cells labelled with daptomycin-bodipy (as described in the materials and methods). The movie shows a series of six optical sections spaced 0.15 microns apart. As the movie progresses, the focal plane moves forward and back through the sections. Dozens of daptomycin-bodipy foci can be seen to surround each of the cells.

**Movie S4.** A 3D reconstruction of a septum labelled with daptomycin-bodipy is rotated 360 degrees about the X-axis.