FIG S1  Time course of BODIPY-FL®-daptomycin penetration measured at the bottom of (a) *S. aureus* 27217, 176 and 33591 and (b) *S. aureus* BCB8 biofilms. The relative intensity corresponds to a normalization of the maximum intensity value to one. Time zero corresponds to the moment at which BODIPY-FL®-daptomycin was added above biofilm.
**FIG S2** Visualization of MSSA and MRSA biofilms using 3D reconstruction to observe biofilm thickness. Images were collected without any drug exposure (control) and after (a) 24 h and (b) 48 h exposure to daptomycin (20 µg/mL) alone and in association with rifampicin (20 µg/mL). Dead cells were stained red with PI and all bacteria were stained green with Syto9®. The acquisition was performed on the whole biofilm thickness with an axial displacement of 1 µm. Images dimension is 82x82µm². The scale bar corresponds to 20 µm.
**FIG S3** Time-kill curves of daptomycin (40 µg/mL) against the four MSSA and MRSA biofilms.

Since no statistically significant difference was observed between the four controls ($P > 0.05$), only one curve was represented. Filled diamonds: controls, filled squares: *S. aureus* 27217, filled triangles: *S. aureus* 176, filled circles: *S. aureus* 33591, crosses: *S. aureus* BCB8. Error bars represent the standard deviation.
Movie S4. Movie sequence of BODIPY-FL®-daptomycin penetration inside *S. aureus* 27217 biofilm obtained by time-lapse imaging showing the fluorescence intensity evolution over time.