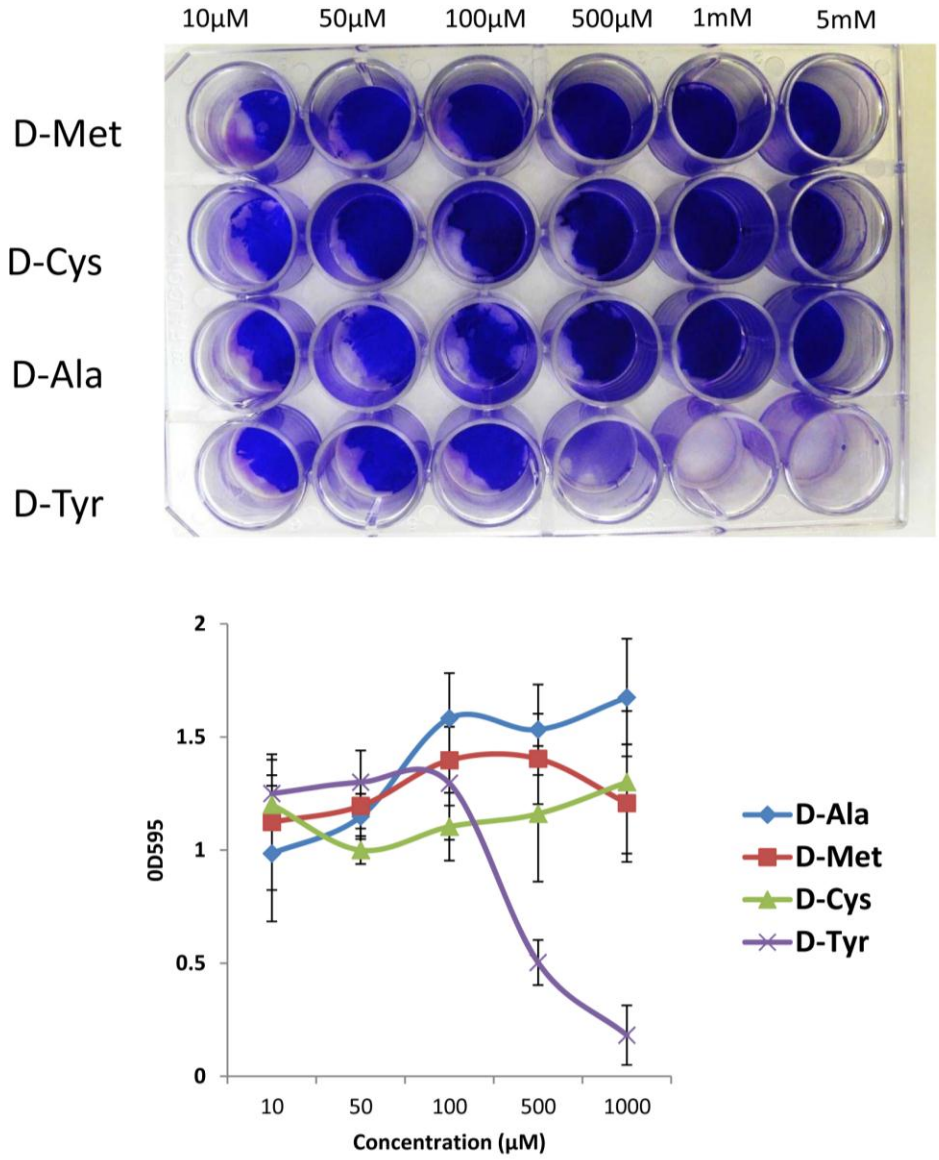

Figure S1. D-Met, D-Cys, D-Ala do not inhibit biofilm formation by *S. aureus*. The medium was supplemented with either D-Met, D-Cys or D-Ala. Biofilm formation by *Staphylococcus aureus* cells was analyzed at 24 h in 12-well polystyrene plates. Quantification of CV staining was done as described in Figure 1, except 100 µl from each well were transferred to 96 wells plate and OD was measured using Synergy 2 platereader (BioTek).

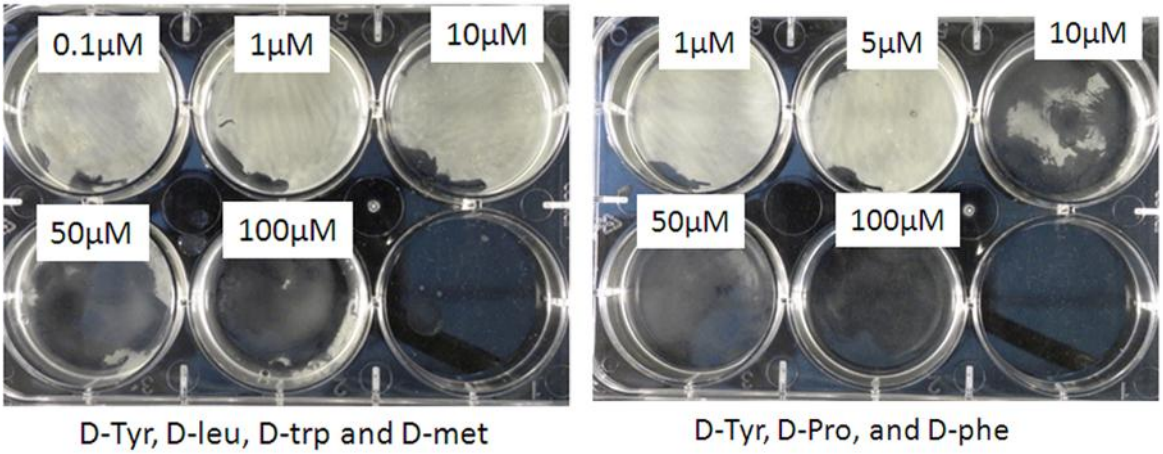

Figure S2. D-Phe, D-Tyr and D-Pro is more effective than D-Trp, D-Tyr, D-Met and D-Leu in inhibiting biofilm formation by *Staphylococcus aureus* cells were grown for 24 h in 12 well polystyrene plates. The medium was supplemented with an equal molar ratio mixture of D-Phe, D-Tyr and D-Pro or with an equal molar ratio mixture of D-Trp, D-Tyr, D-Met and D-Leu. In contrast to *B. subtilis*, *S. aureus* biofilm growth is more sensitive to the D-Phe, D-Tyr and D-Pro mixture.

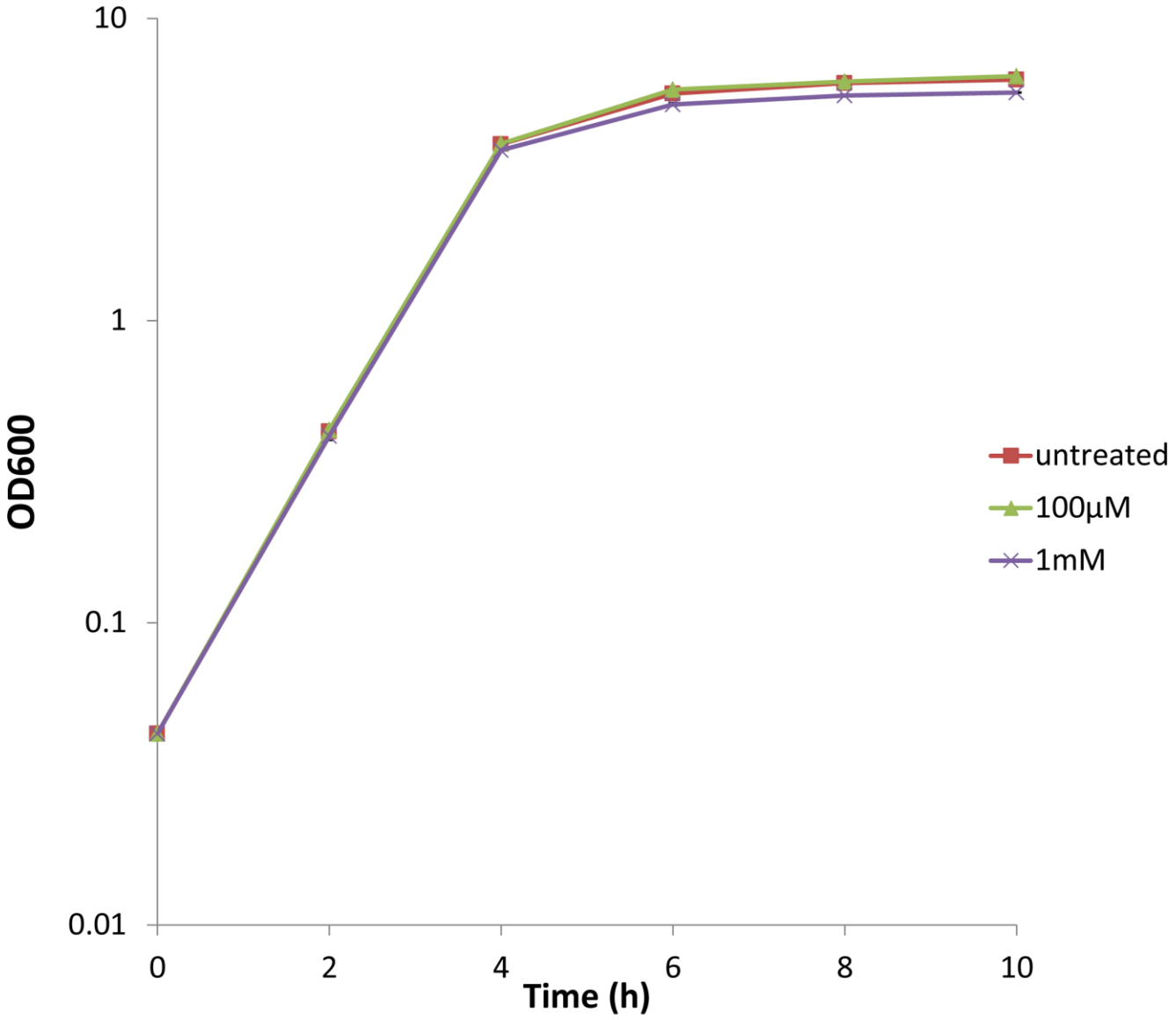

FIGURE S3. D-aa mixture does not inhibit cell growth. *S. aureus* SC01 was grown overnight in LB at 37°C and inoculated into TSB containing 3% NaCl and 0.5% glucose at a staring optical density of 0.05 in 250ml baffled flasks. An equimolar mixture of D-tyrosine, D-proline and D-tryptophan were added to final concentrations of 0 µM (squares), 100 µM (triangles) or 1 mM (crosses) in duplicate flasks. Cultures were grown at 37°C with shaking for 10 hours and the OD600 measured every two hours. The average of two replicates is shown.

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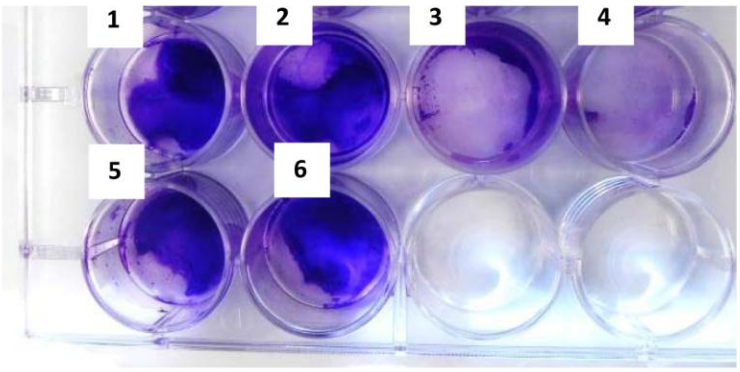

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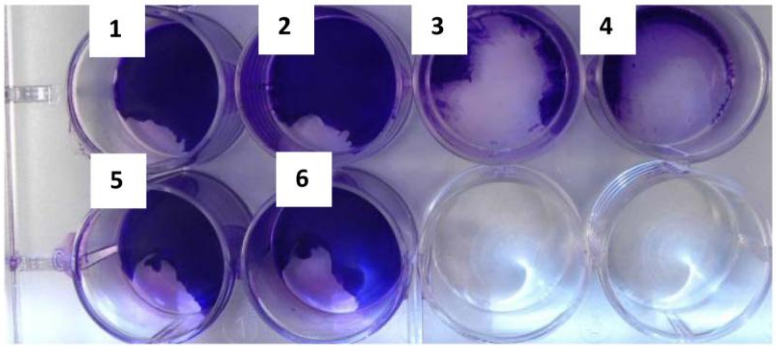

1) Untreated
2) D-aa: 10µM
3) D-aa: 100µM
4) D-aa: 500µM
5) L-aa:100µM
6) L-aa:500µM

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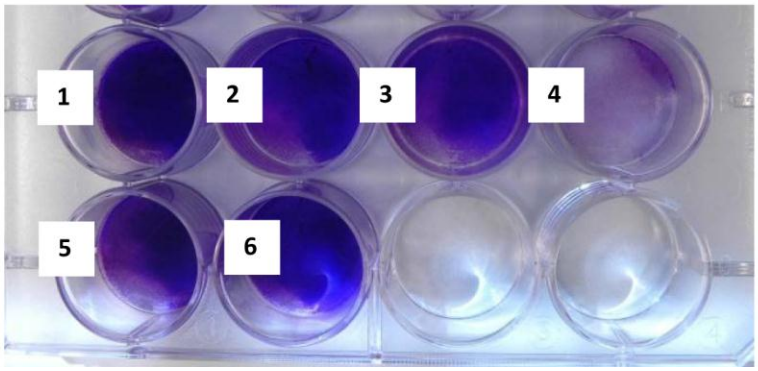


Figure S4. D-Phe, D-Tyr and D-Pro inhibit biofilm formation by multiple *Staphylococcus aureus* strains. The medium was supplemented with an equal molar ratio mixture of D-Phe,
D-Tyr and D-Pro, or an equal molar ratio mixture of the equivalent L-amino acids.

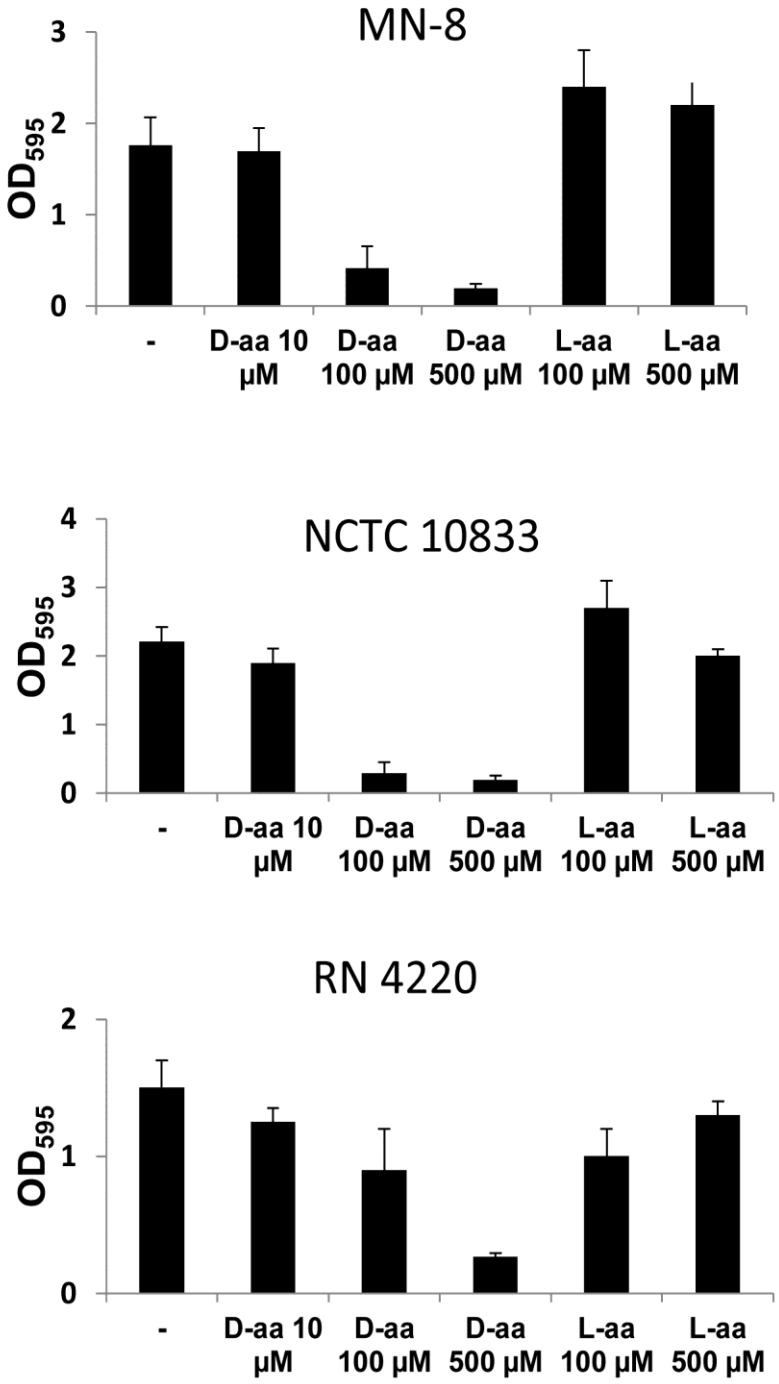


FIGURE S5. Quantification of the effects of D-Phe, D-Tyr and D-Pro in inhibiting biofilm formation by multiple *Staphylococcus aureus* strains. The medium was supplemented with an equal molar ratio mixture of D-Phe, D-Tyr and D-Pro. Quantification of CV staining was done as described in Figure 1, except 100µl from each well were transferred to 96 wells plate and OD was measured using Synergy 2 plate reader (BioTek). The results represent the average of 3 independent experiments each performed in triplicate.

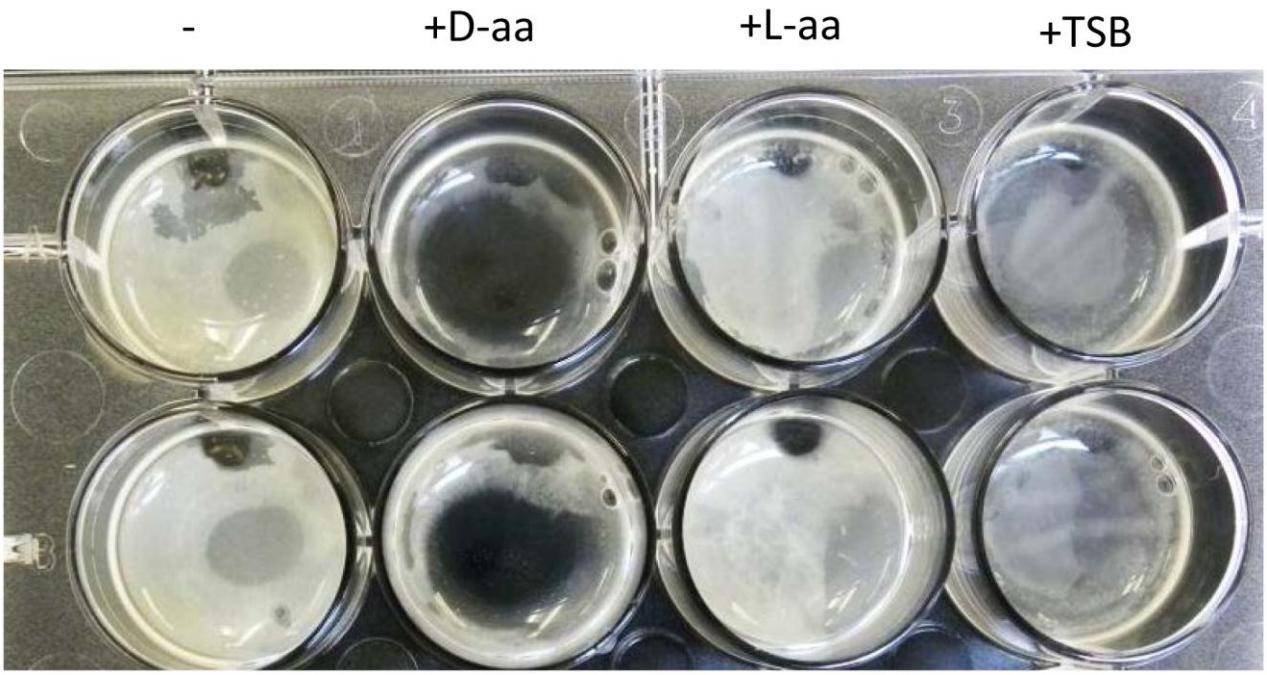
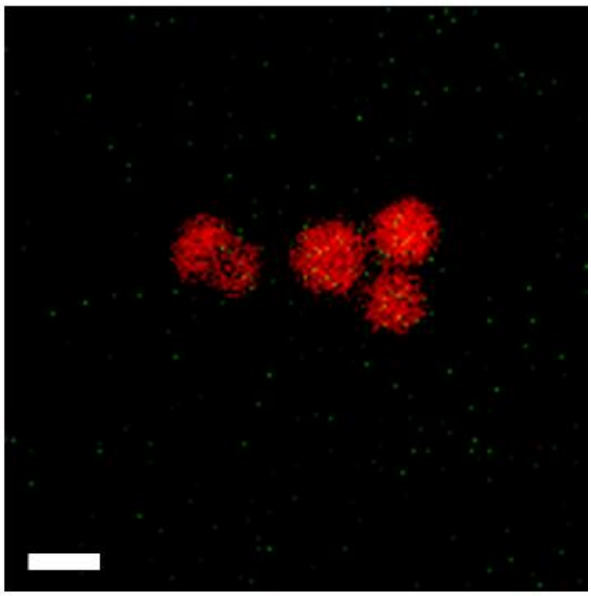


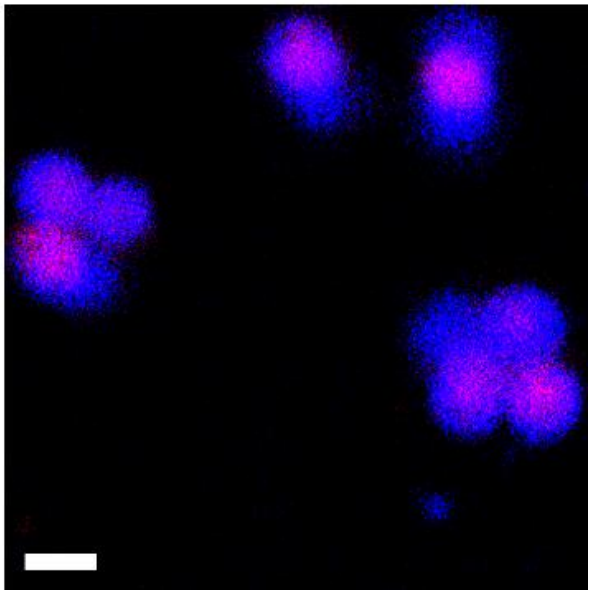
FIGURE S6. D-Phe, D-Tyr and D-Pro cause biofilm disassembly by *Staphylococcus* at high concentration. *Staphylococcus aureus* cells were grown for 24 h in 12 well polystyrene plates. After 24 hr, the medium was either not replaced (-), or replaced with either TSB containing 10 mM of the D-aa mixture (+D-aa), TSB containing 10 mM of the L-aa mixture (+L-aa), or with unsupplemented TSB (+TSB). Plates were incubated for additional 48 hrs. Medium was removed and wells were treated as described in Materials and Methods.

A)



High magnification of D-aa treated cells stained for extracellular protein (green) and DNA (red)

B)



High magnification of D-aa treated cells stained for exopolysaccharides (blue) and DNA (red)

FIGURE S7. High magnification of D-aa treated cells shown in Figure 4 (A) and 5 (B)

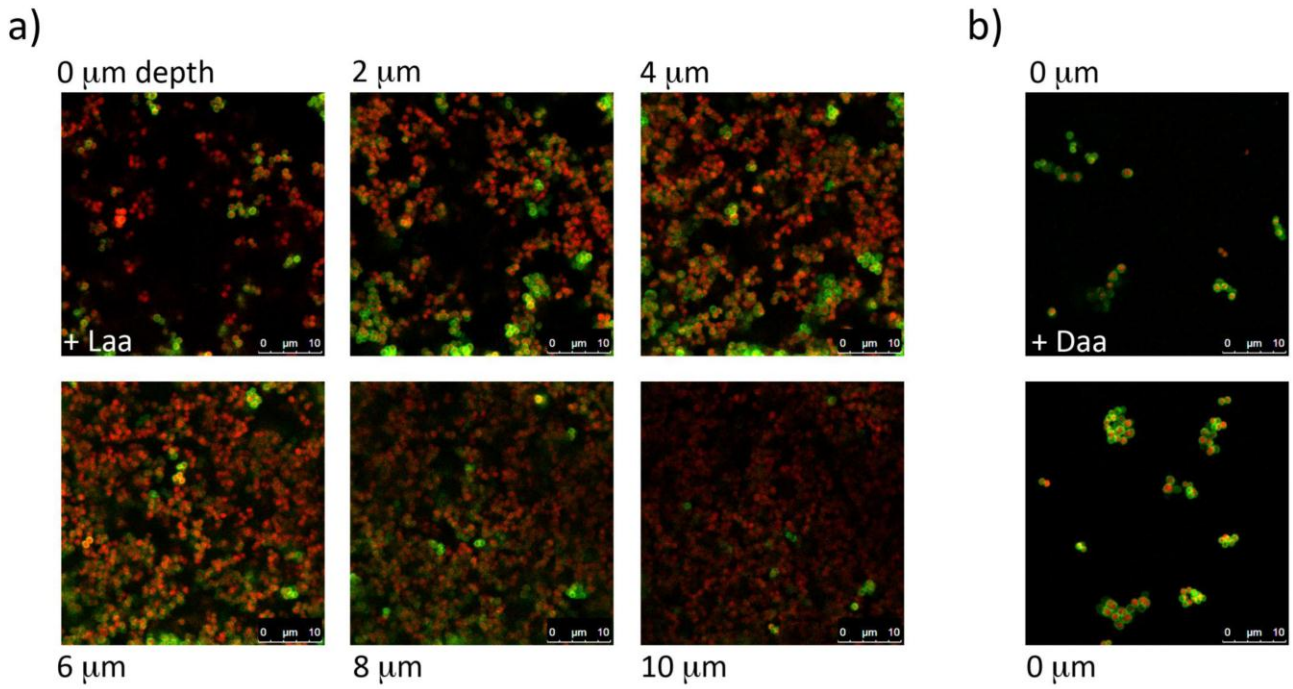


FIGURE S8. D-aa do not affect exopoplysacchride localization as examined by WGA staining. Using the same protocol as in figure 5, Wheat germ agglutinin **(**WGA**)** was applied to the final concentration of 5μg/ml together with SYTO63.

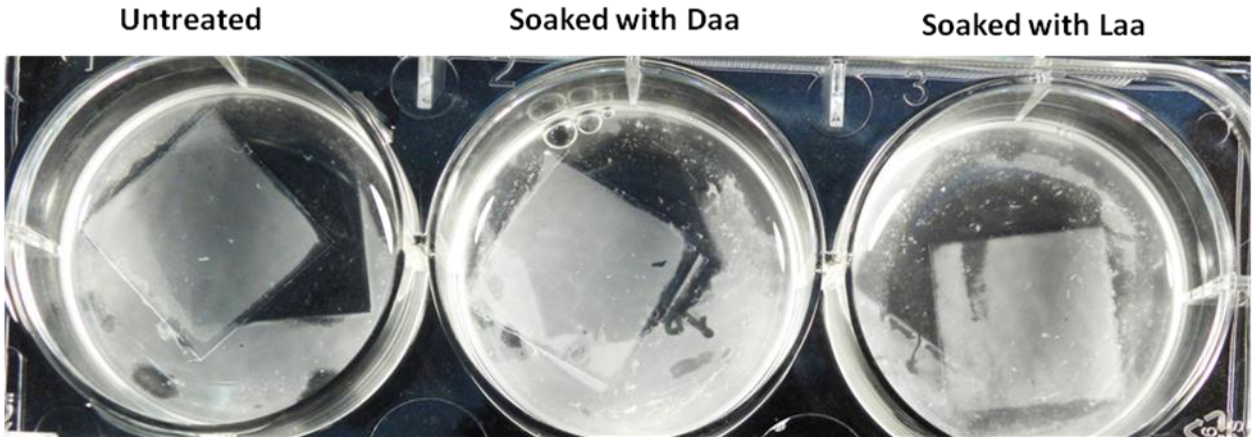


FIGURE S9. Negative control for D-aa soaked polymer substrates inhibiting biofilm growth. Glass substrates that had been soaked with D-aa do not absorb D-aa and therefore do not inhibit biofilm growth.