Supporting Information

Metabolic glycoengineering of *Staphylococcus aureus* reduces adherence to human T24 bladder carcinoma cells

E. Memmel, A. Homann†, T. A. Oelschlaeger, J. Seibel*

Methods

Cultivation of T24 cells

T24 human bladder carcinoma cells were cultivated in McCoy's 5A medium (PAA) containing 2mM L-glutamine and 10% fetal calf serum (FCS) at 37 °C in a 5% CO_2 atmosphere. At 80-90% confluence the medium was discarded and the cells were washed with phosphate buffered saline (PBS, pH 7.0, Gibco). By adding 5 ml of a trypsin/EDTA solution (PAA) the cells were detached for 5 min at 37 °C. 5 ml of fresh medium was added and the cells were split at a ratio of 1:10 for up to 20 passages. For adhesion experiments, T24 cells were cultivated as described above. At 80-90% confluency the cells were seeded into 8-well microscopy cultivation chamber slides (ibidi) at a split ratio of 1:1 and a final volume of 300 µl per well. The cells were incubated until reattachment (approximately 18 h).

Metabolic glycoengineering and bioorthogonal labeling of *Staphylococcus aureus*

S. aureus 8325 was cultivated in B medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.1% K_2HPO_4, 0.1% glucose, pH 7.2) at 37 °C overnight. 20 µl of the culture were diluted with fresh medium to a final volume of 2 ml and the bacteria were grown for two hours. The suspensions were diluted to an OD_{600} of 0.1 and supplemented with 2-acetylamino-2-deoxy-β-D-glucopyranoside (GlcNAc) or 2-azidoacetylamino-2-deoxy-β-D-glucopyranoside (GlcNAz) at a final concentration of 10 mM. GlcNAz was synthesized as described previously.[1] The cells were grown for another two hours at 37 °C, after which they were sedimented by centrifugation (5 min, 3000 g), resuspended and washed twice with PBS buffer (pH 7.0) and diluted to an OD_{600} of 0.1 with the same buffer.
For the click reaction based labeling in PBS (pH 7.0), $CuSO_4$ (200 µM), Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA, 200 µM), sodium ascorbate (400 µM) and alkynylated Alexa-488 (10 µM) were added in this order. *S. aureus* was incubated at room temperature in the dark for one hour, subsequently washed several times with PBS, followed by PBS/DMSO 1:1 mixture. The cells were diluted to a final OD_{600} of 0.1 with PBS (pH 7.0) and used for the adhesion experiments. Where indicated, the commercial TAMRA-based click-labeling kit was used as described by the manufacturer (Click-IT® Tetramethylrhodamine (TAMRA) Protein Analysis Detection Kit, Molecular Probes). Controls were performed after GlcNAc/GlcNAz incubation without additional reagents (termed "buffer" in figure 3), $CuSO_4$, TBTA and sodium ascorbate ("click" in figure 3) or alkynylated Alexa-488 only ("alkyne" in figure 3).

Adhesion assay of *Staphylococcus aureus* to human T24 bladder cancer cells

To characterize the effect of metabolic glycoengineering on the adhesion process, 8 µl of bacteria suspension (OD_{600} 0.1 as descibed above) was added per well of the microscopy cultivation slide and incubated at 37 °C for two hours. The medium was discarded and the cells were washed with 200 µl McCoy's 5A medium three times to remove unattached bacteria. After fixation with methanol the cells were examined by bright field and fluorescence microscopy (580 nm for TAMRA and 525 nm for Alexa-488). Adherent bacteria were counted in five fields of vision per well located in the four corners and the middle of the area.

Figures

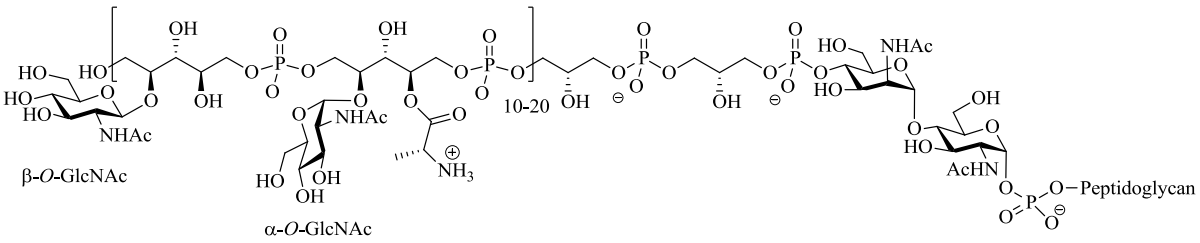

Fig. S1: General structure of wall teichoic acid (*S. aureus*).

Literature

1. S. J. Luchansky, H. C. Hang, E. Saxon, J. R. Grunwell, C. Yu, D. H. Dube and C. R. Bertozzi, *Methods Enzymol*, 2003, **362**, 249-272.