Electronic Supplementary Material (ESI) for Analyst.

Spatial Organization of *Pseudomonas aeruginosa* Biofilms Probed by Combined Laser Desorption Ionization Mass Spectrometry and Confocal Raman Microscopy

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Supplemental Information

Fluorescence Imaging of Biofilms. Biofilms were developed for 72 h on bare Si and Au-coated Si substrates as described in the Experimental section. Hydrated biofilms on both Au-coated and bare Si substrates were stained with nucleic acid specific SYTO 24 (Molecular probes), which is cell permeable. SYTO 24 dye was added and incubated for 20 min before fluorescence imaging. Fluorescence images were acquired using a Nikon AR-1 confocal laser scanning microscope using a 100x, 0.9 NA Nikon objective at an excitation wavelength of 488 nm. Images showing the cells found in the wild type *P. aeruginosa* and mutant biofilms on Au-coated and bare Si substrates are shown in Figure S1 below.

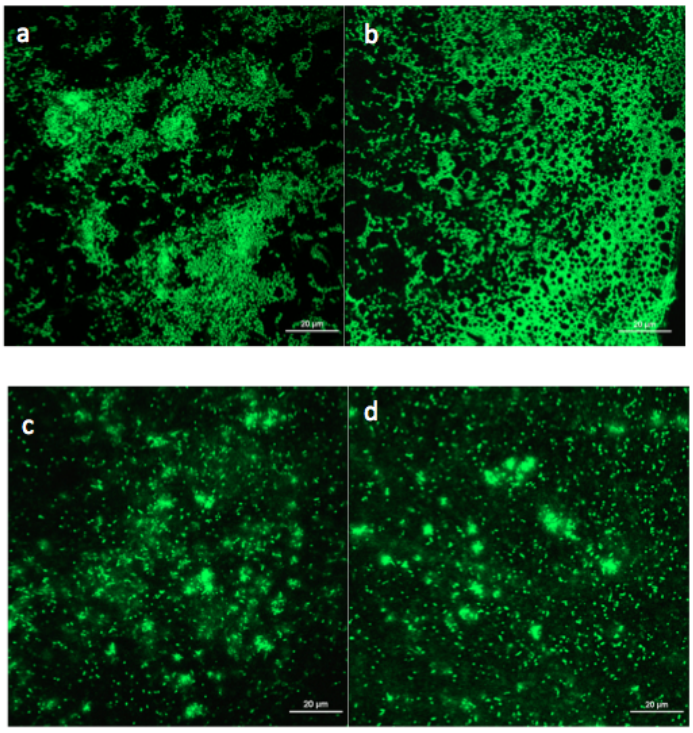


Figure S1. Fluorescence images of wild type biofilms on bare silicon wafer (a), and on gold-coated silicon wafer (b), and mutants prepared under biofilm conditions on bare silicon wafer (c), and gold-coated silicon wafer (d). Biofilms were stained with SYTO 24.

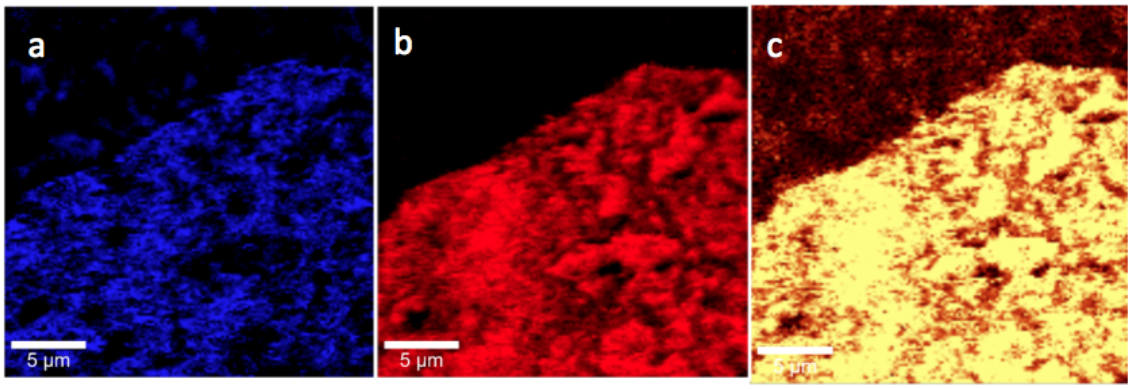


Figure S2. Confocal Raman images showing the distribution of carbohydrates and glycolipids (1010 -1165cm^{-1}), blue (a), proteins (1560-1620 cm^{-1}), red (b), and all organic matter (2800-3050 cm^{-1}) yellow (c).

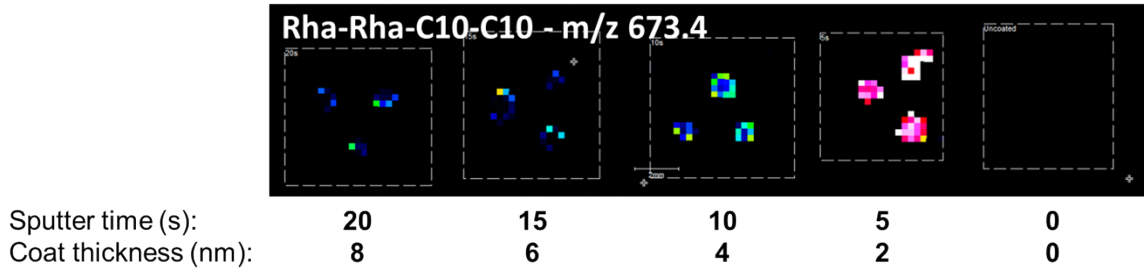
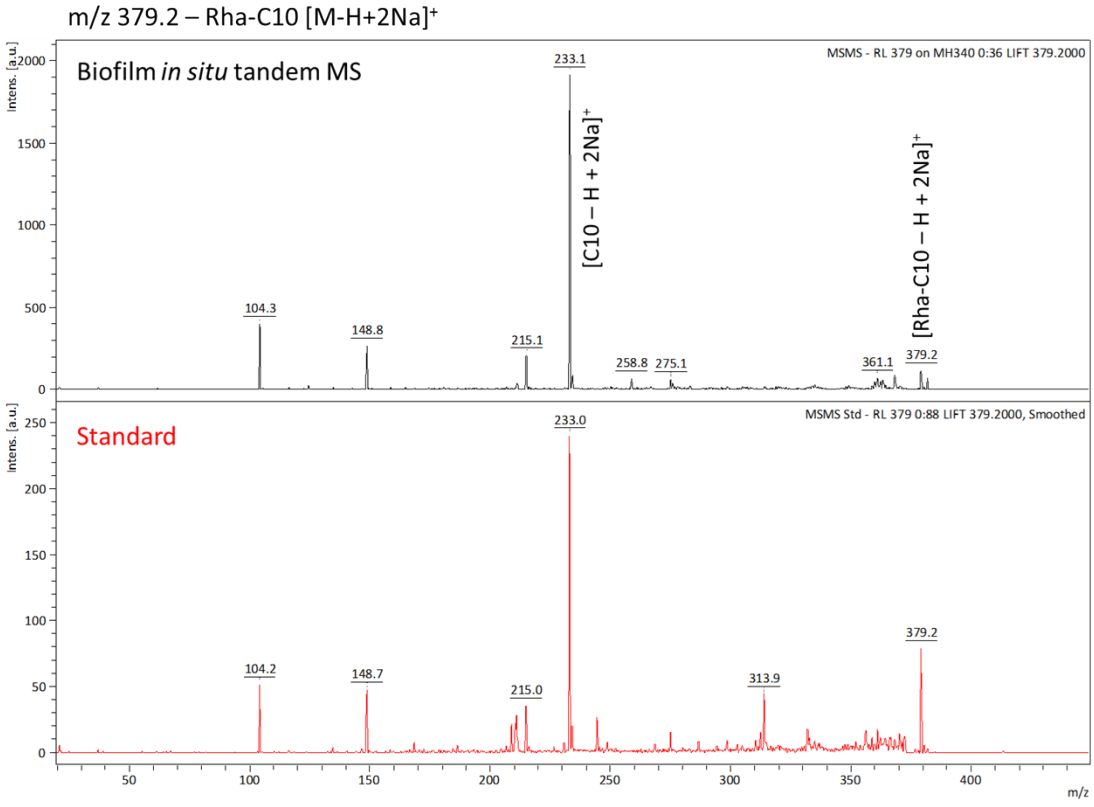
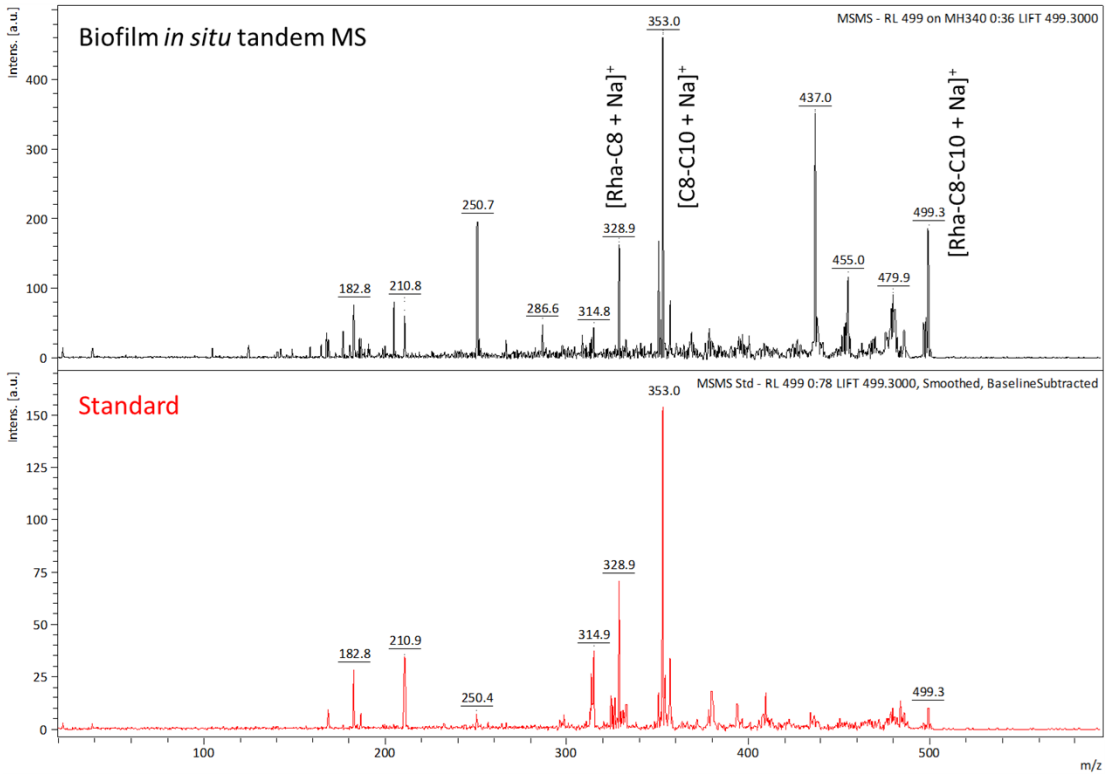


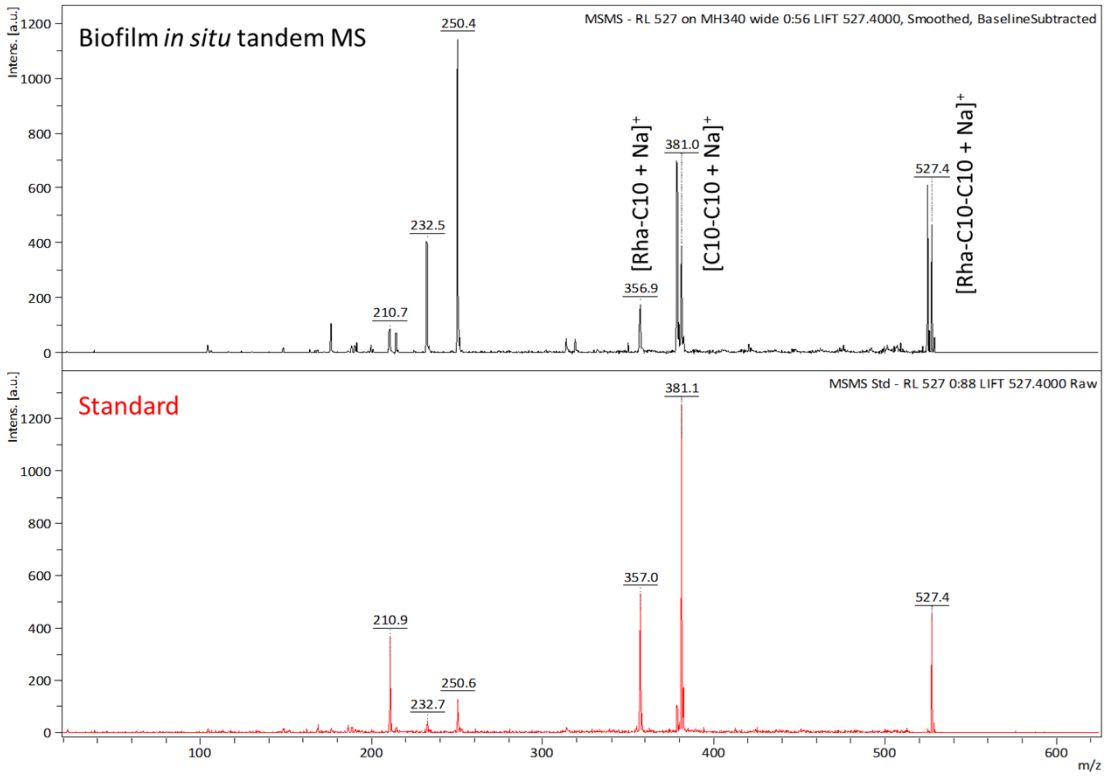
Figure S3. Optimization of Au sputter coat thickness for MALDI MSI. Rhamnolipid standard was spotted on silicon wafer substrates and sputter-coated for 0-20 seconds, corresponding to a 0-8 nm-thick Au coating. The ion image of the predominant rhamnolipid species Rha-Rha-C10-C10 (shown here) shows greatest signal with the thinnest (~2 nm) coating.



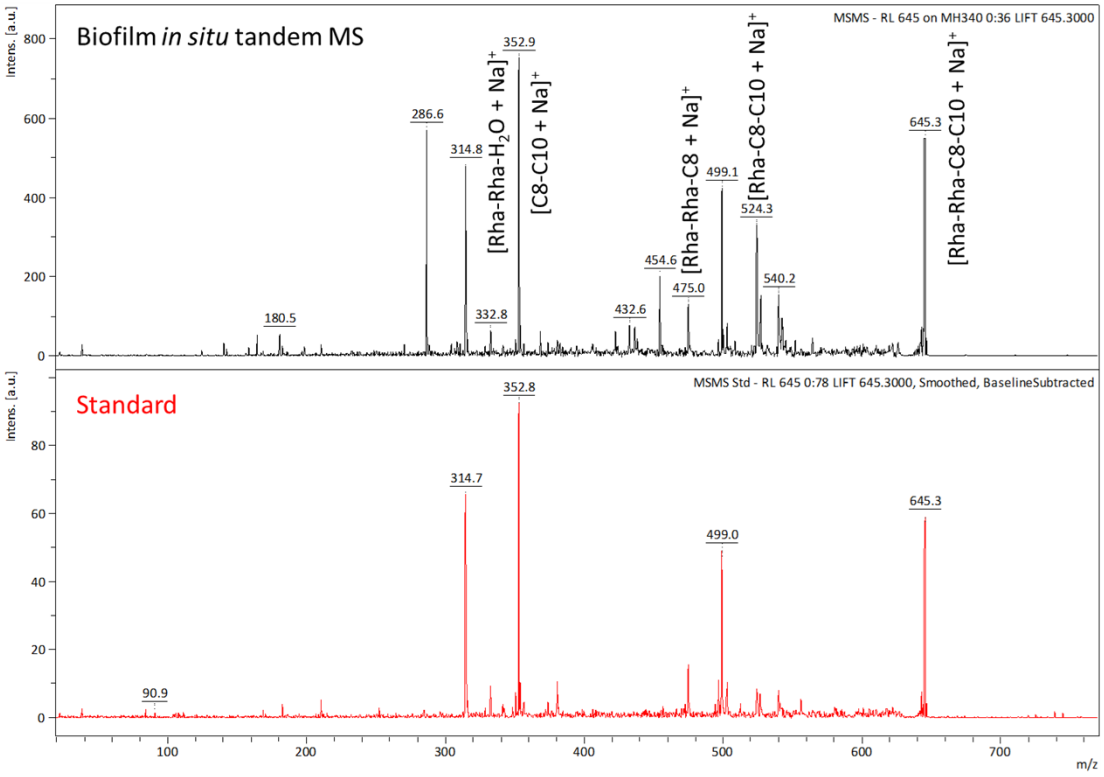
m/z 499.3 – Rha-C8-C10 [M+Na]$^+$



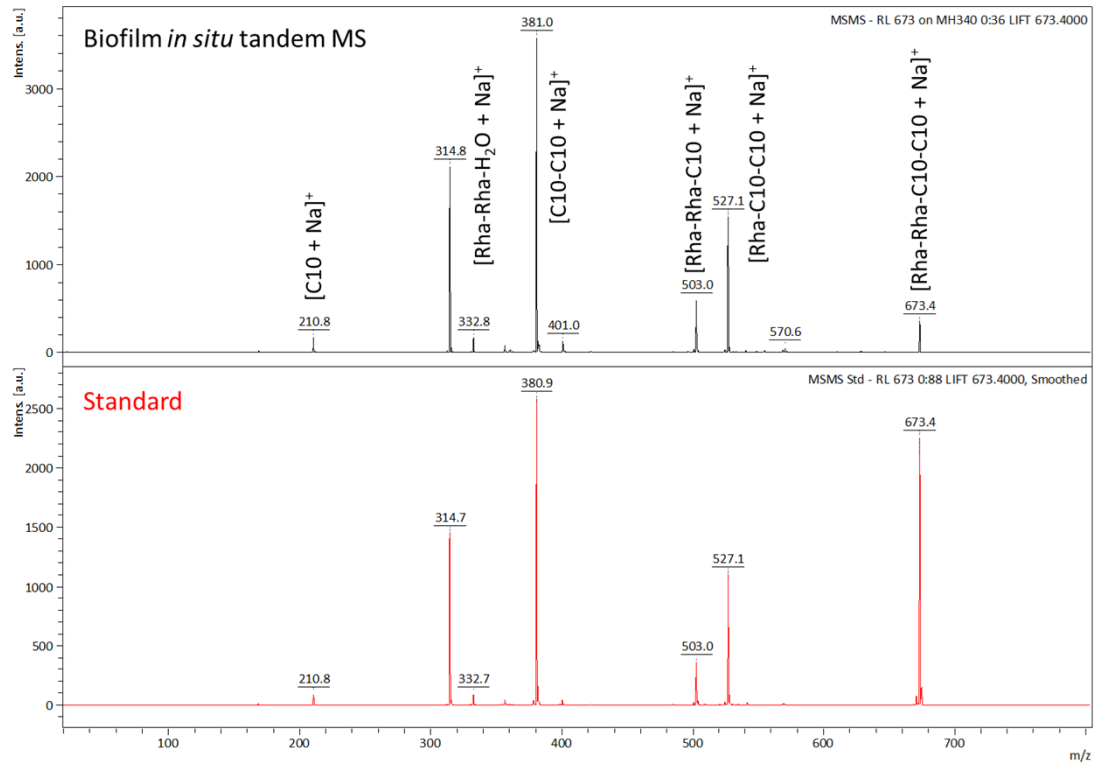
m/z 527.3 – Rha-C10-C10 [M+Na]$^+$



m/z 645.3 – Rha-Rha-C8-C10 [M+Na]$^+$



m/z 673.4 – Rha-Rha-C10-C10 [M+Na]$^+$



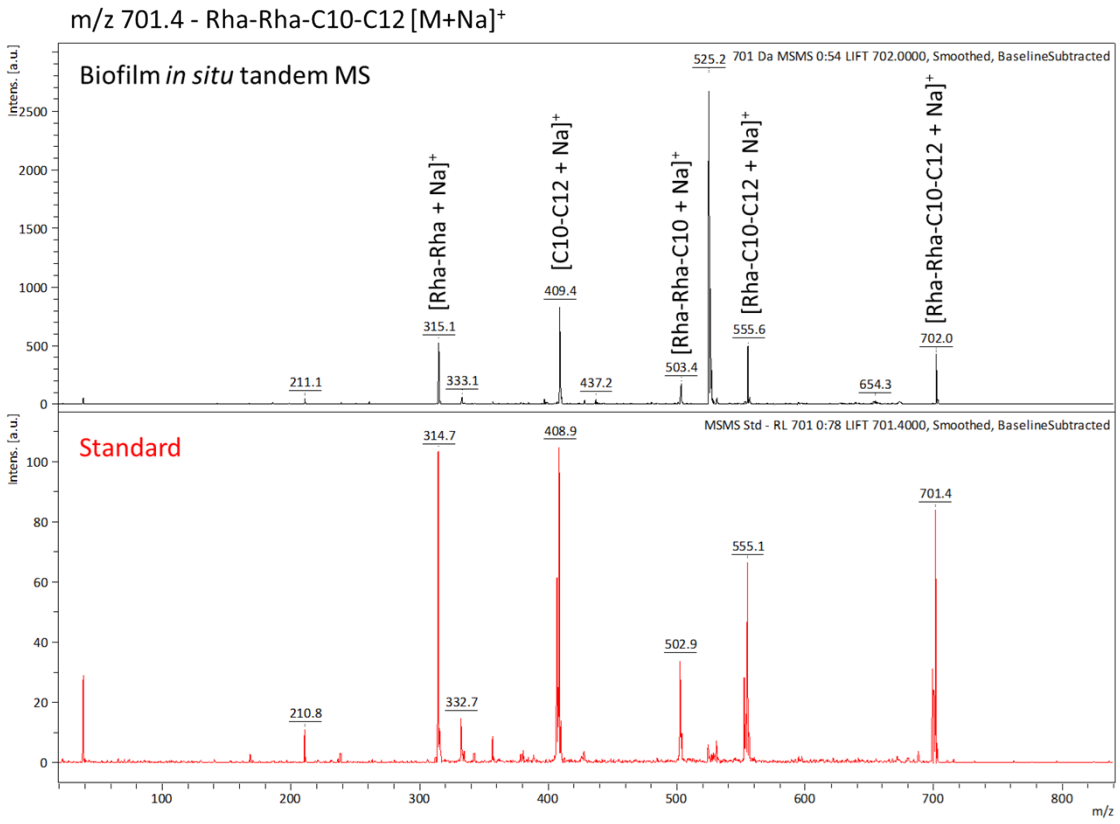


Figure S4. Tandem MS spectra for rhamnolipid-related ions, acquired *in situ* by MALDI-TOF/TOF MS on wild type *P. aeruginosa* biofilm (top) and from mixed rhamnolipid standard (bottom), confirm mass assignments. Characteristic fragments are matched to and in agreement with previous MS/MS analyses (see ref. 74 – de Koster et al. (1994) Bio Mass Spec 23, 179). Additional unassigned fragments may arise from post-source decay-specific fragmentation pathways not produced in CID and/or fragmentation of other nearly isobaric ions which were necessarily included in the parent isolation window.