

## **Antibacterial properties of glycosylated surfaces: variation of the glucosidal moiety and fatty acid conformation of grafted microbial glycolipids**

### **Supplementary information**

Claire Valotteau<sup>1,2</sup>, Sophie Roelants<sup>3,4</sup>, Prabhu Dasaiyan<sup>5</sup>, Susanne Zibek<sup>6</sup>, Michael Günther<sup>7</sup>, Wim Soetaert<sup>3,4</sup>, Bernd Everaert<sup>4</sup>, Claire-Marie Pradier<sup>1</sup>, Florence Babonneau<sup>2</sup>, Niki Baccile<sup>2,§</sup>, Vincent Humblot<sup>1,#,§</sup>.

<sup>1</sup> Sorbonne Université, Laboratoire de Réactivité de Surface (LRS), UMR CNRS 7197, 4 place Jussieu, Paris, F-75005, France.

<sup>2</sup> Sorbonne Université, Laboratoire de Chimie de la Matière Condensée de Paris (LCMCP), UMR CNRS 7574, 4 place Jussieu, Paris, F-75005, France.

<sup>3</sup> Ghent University, Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Coupure Links 653, B-9000 Gent, Belgium

<sup>4</sup> Bio Base Europe Pilot Plant, Rodenhuzekaai 1, B-9000 Gent, Belgium

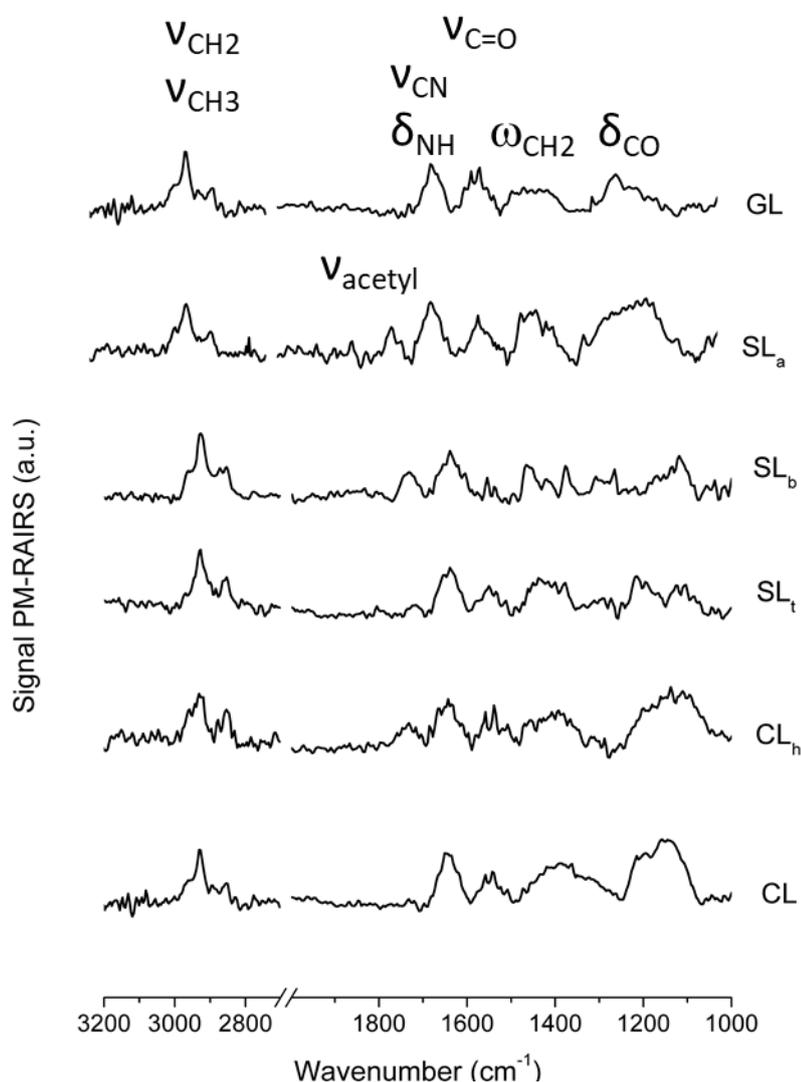
<sup>5</sup> Physical and Materials Chemistry Division, CSIR - National Chemical Laboratory, Pune - 411 008, India

<sup>6</sup> Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Nobelstraße 12, D-70569 Stuttgart, Germany

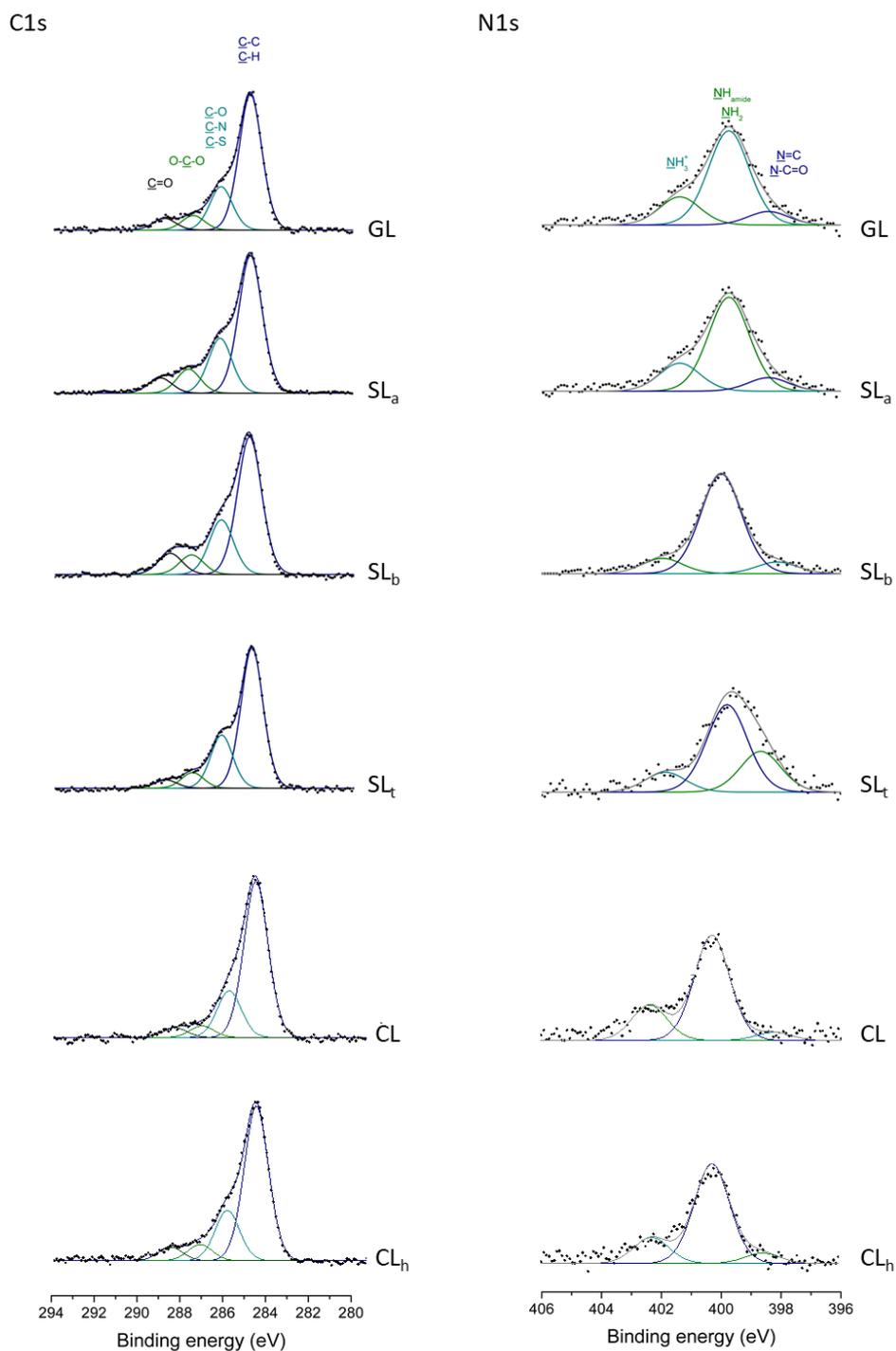
<sup>7</sup> Universität Stuttgart, Institut für Grenzflächenverfahrenstechnik, Nobelstraße 12, D-70569 Stuttgart, Germany

# present address: FEMTO-ST Institute, UMR CNRS 6174, Université Bourgogne Franche-Comté, 15B avenue des Montboucons, 25030 Besançon Cedex, France.

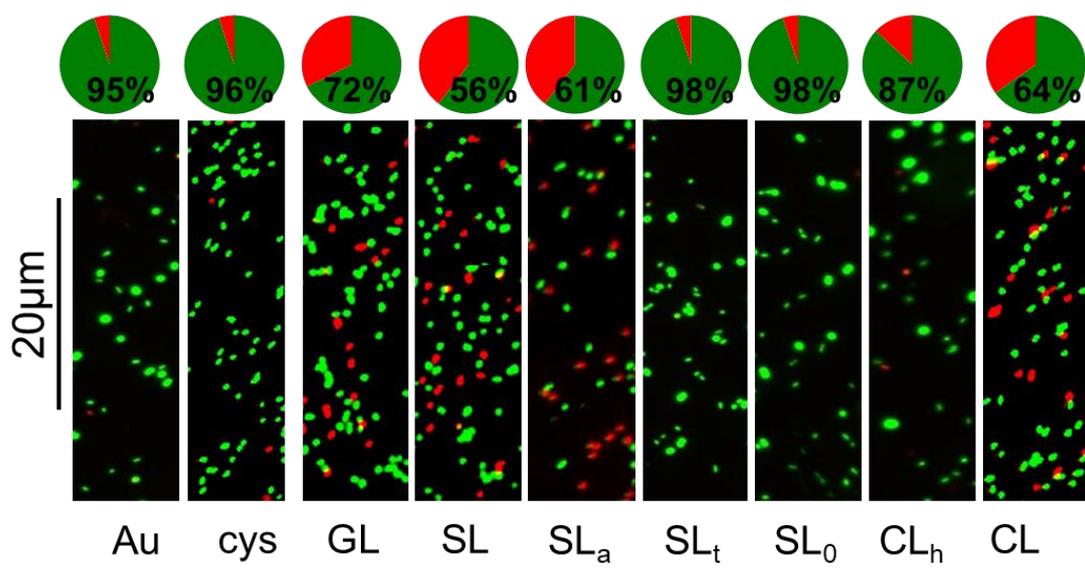
§Corresponding authors: niki.baccile@sorbonne-universite.fr; vincent.humblot@femto-st.fr



**Figure S1.** PM-RAIRS spectra of self-assembled monolayers of glucolipid (GL), acetylated *cis* sophorolipid (SL<sub>a</sub>), branched sophorolipid (SL<sub>b</sub>), *trans* sophorolipid (SL<sub>t</sub>), cellobioselipid (CL) and hydrolyzed cellobioselipid (CL<sub>h</sub>). The resonance signal at 1648 cm<sup>-1</sup> ( $\nu_{\text{C=O}}$  in amide) and 1562 cm<sup>-1</sup> ( $\nu_{\text{CN}}$  and  $\delta_{\text{NH}}$ ) are characteristic of amide bonds. The presence of aliphatic chains is attested by vibrational bands at 3000-2800 cm<sup>-1</sup> ( $\nu_{\text{S}}$  and  $\nu_{\text{AS}}$  of CH<sub>2</sub> and  $\nu_{\text{S}}$  of CH<sub>3</sub>) and 1400 cm<sup>-1</sup> ( $\omega_{\text{CH}_2}$ ). The signal of carbohydrates resonates at 1200-1000 cm<sup>-1</sup> ( $\delta_{\text{CO}}$ ), where a multitude of bands are partially overlapped and therefore difficult to interpret in detail. The elongation of the C=O bonds on acetyl moieties produces an additional band at 1740 cm<sup>-1</sup> on spectra of samples prepared with acetylated SL<sub>a</sub> and CL.



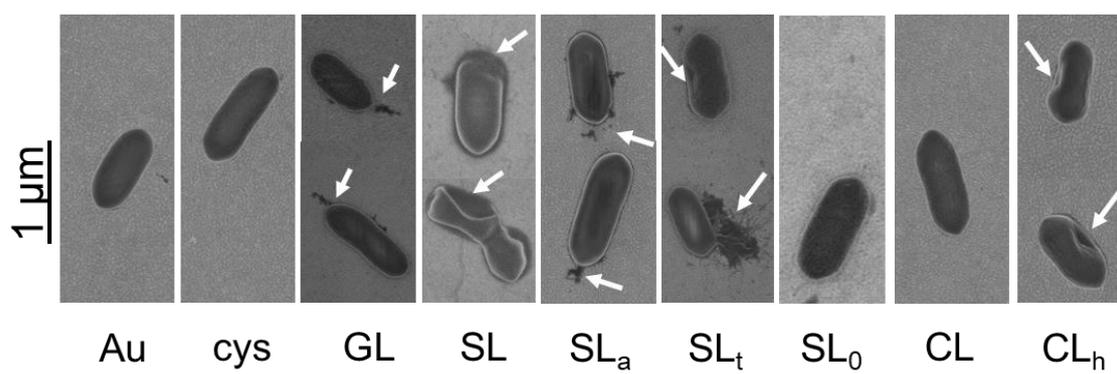
**Figure S2.** High resolution standardized XPS spectra of the C1s (left) and N1s (right) regions recorded on self-assembled monolayers of glucolipid (GL), acetylated *cis* sophorolipid (SL<sub>a</sub>), branched sophorolipid (SL<sub>b</sub>), *trans* sophorolipid (SL<sub>t</sub>), cellobioselipid (CL) and hydrolyzed cellobioselipid (CL<sub>h</sub>). The peak at  $284.8 \pm 0.1$  eV in the C1s region attests the presence of an aliphatic chains while the two contributions, at  $286.4 \pm 0.1$  eV (C-OH) and  $287.7 \pm 0.1$  eV (O-C-O) are the signature of the carbohydrate groups, also visible in the O1s region. The shift of the nitrogen signal from  $401.8 \pm 0.1$  eV (NH<sub>3</sub><sup>+</sup>) on cys primer layer towards  $399.9 \pm 0.1$  eV (NH<sub>2</sub> and NH in amide) after immersion on glycolipids solution demonstrate that the glycolipids are grafted via amide bonds.



**Figure S3.** Fluorescent staining evidences membrane damages of bacteria (*L. ivanovii*) deposited on self-assembled monolayers of glucolipid (GL), deacetylated *cis* sophorolipid (SL), acetylated *cis* sophorolipid (SL<sub>a</sub>), *trans* sophorolipid (SL<sub>t</sub>), saturated sophorolipid (SL<sub>0</sub>), hydrolyzed cellobioselipid (CL<sub>h</sub>) and cellobioselipid (CL).

Biocompatible gold (Au) surfaces and cysteamine (cys) monolayers constitute negative (glycolipid-free) controls.

The chart above each image represents the proportion of adhering intact (percentage given) and damaged bacteria according to fluorescent staining.



**Figure S4.** Scanning electron microscopy reveals qualitative morphological alterations (highlighted by white arrows) of bacteria (*L. ivanovii*) deposited on self-assembled monolayers of glucolipid (GL), deacetylated *cis* sophorolipid (SL), acetylated *cis* sophorolipid (SL<sub>a</sub>), *trans* sophorolipid (SL<sub>t</sub>), saturated sophorolipid (SL<sub>0</sub>), cellobioselipid (CL) and hydrolyzed cellobioselipid (CL<sub>h</sub>). Biocompatible gold (Au) surfaces and cysteamine (cys) monolayers constitute negative (glycolipid-free) controls.