Supplementary files

**Membrane-partitioned cell wall synthesis in mycobacteria**

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| **Supplementary file 1a. Incubation conditions** | | |
| Bacterium | Antibiotic or compound | Incubation conditions |
| *M. smegmatis* | 100 mM benzyl alcohol | 60 min at 37ºC with shaking |
|  | 0.5 mM dibucaine | 180 min at 37ºC with shaking |
| DivIVA-eGFP-ID | 50 ng/mL ATC | 8 h, 14 to 16 h |
| MurJ-ID |  | 8h |
| *B. subtilis* | 24 µg/mL kanamycin | 10 min at 37°C with shaking |
| 50 µg/mL phosphomycin |
| 50 mM benzyl alcohol |
| *C. crescentus* | 5 µg/mL kanamycin | 30 min at 30°C with shaking |
| 50 µg/mL phosphomycin |
| 50 mM benzyl alcohol |
| *E. coli* | 20 µg/mL A22 | 90 min at 37°C with shaking. Cultures were back-diluted after 40 min of incubation to an OD600 ~0.3-0.5 in prewarmed medium + A22 and incubated until completion of 90 min |
| 16 µg/mL kanamycin | 30 min at 37ºC with shaking |
| 50 µg/mL phosphomycin |
| 50 mM benzyl alcohol |
| *S. aureus* and *L. lactis* | 50µg/mL phosphomycin | 30 min at 37ºC with shaking |
| 20 µg/mL kanamycin  50 mM benzyl alcohol |
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| 50 µg/mL ampicillin (with 5 µg/mL clavulanate) |

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| **Supplementary file 1b. Metabolic labeling conditions** | | |
| Bacterium | Probe | Labeling conditions |
| *M. smegmatis* | alkDADA  N-Alk-TMM  O-Alk-TMM | Unless otherwise indicated, 15 min with shaking at 37ºC |
| *E. coli*  *B. subtilis*  *S. aureus*  *L. lactis* | alkDADA | Unless otherwise indicated, 5 min with shaking at 37ºC |
| *C. crescentus* | 5 min with shaking at 30ºC |

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| **Supplementary file 1c. NCBI Accession numbers from 16S rDNA** | |
| Bacterium | NCBI accession number |
| *M. smegmatis* | AB305022.1 |
| *B. subtilis* | NR\_112116.2 |
| *S. aureus* | NR\_118997.2 |
| *L. lactis* | FJ348447.1 |
| *E. coli* | NR\_024570.1 |
| *C. crescentus* | NR\_037099.1 |