

Legends of the Supplementary Figures

Figure S1. 1-D SDS-PAGE bands analyzed by MS/MS

The extracted membrane protein fraction was separated by 1-D SDS-PAGE according to Laemmli [43] using a 12% acrylamide gel with a length of 16 cm, followed by CBB G250 staining. The protein bands that were analyzed by LC-MS/MS and MALDI-MS/MS are labeled. These bands are also annotated in our mycobacterial proteome database, which is publicly accessible at <http://www.mpiib-berlin.mpg.de/2D-PAGE/>.

Figure S2. 2-DE spots analyzed by MS/MS

The extracted membrane protein fraction was separated by 2-DE according to Klose and Kobalz [46] using a 15% acrylamide gel with a size of 6.6 × 8.0 cm. The depicted analytical gel was silver stained. The protein spots that were analyzed by MALDI-MS/MS are labeled. These spots are also annotated in our mycobacterial proteome database, which is publicly accessible at <http://www.mpiib-berlin.mpg.de/2D-PAGE/>.

Figure S3. Isoelectric points and masses of the identified proteins

The figure shows the pI and mass distribution of the 3,924 *M. tuberculosis* H37Rv proteins described by Cole et al. [4] (A), of the 787 predicted *M. tuberculosis* H37Rv membrane proteins (B), and of the proteins identified in this study by either 1-D SDS-PAGE- (black dots) or 2-DE-based (white triangles) proteome analysis of the purified membrane protein fraction (C). The figure illustrates the finding that certain integral membrane proteins of *M. tuberculosis* H37Rv show extremes in pI and/or mass and were thus not accessible to our analyses.

Figure S4. Relation between the number of TMD and GRAVY of mycobacterial proteins

The GRAVY scores of the 3,924 *M. tuberculosis* H37Rv proteins described by Cole et al. [4] were determined according to Kyte and Doolittle [63] and plotted with respect to the number of predicted TMD. Note that each data point represents a single protein. In some cases individual markings merge. Orange triangles denote the average GRAVY scores of proteins with equal numbers of predicted TMD. The figure illustrates the finding that proteins with equal numbers of TMD show strongly varying GRAVY scores. Thus, the GRAVY should not be used as the only index for determination of the recovery of membrane proteins.

Figure S5. GRAVY scores of the identified proteins

The figure shows the GRAVY distribution of the 3,924 *M. tuberculosis* H37Rv proteins described by Cole et al. [4] (black bars) and of the proteins identified in this study by either 1-D SDS-PAGE- (orange bars) or 2-DE-based (white bars) proteome analysis of the extracted membrane protein fraction. Note that for reasons of comprehensibility the labels of the GRAVY intervals were simplified, e.g. -1.5 to -1.4 literally means ≥ -1.5 but < -1.4 . The figure illustrates that the 1-D SDS-PAGE-based proteome analysis of the membrane protein fraction revealed numerous proteins with a strongly hydrophobic character including several proteins with a GRAVY $> +0.5$, whereas the 2-DE-based analysis of this fraction failed to identify such proteins.