P116 from Mycoplasma is a self-sufficient lipid uptake and delivery machinery

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Extended Data Table 1: Statistical analysis of liposome fluorescence

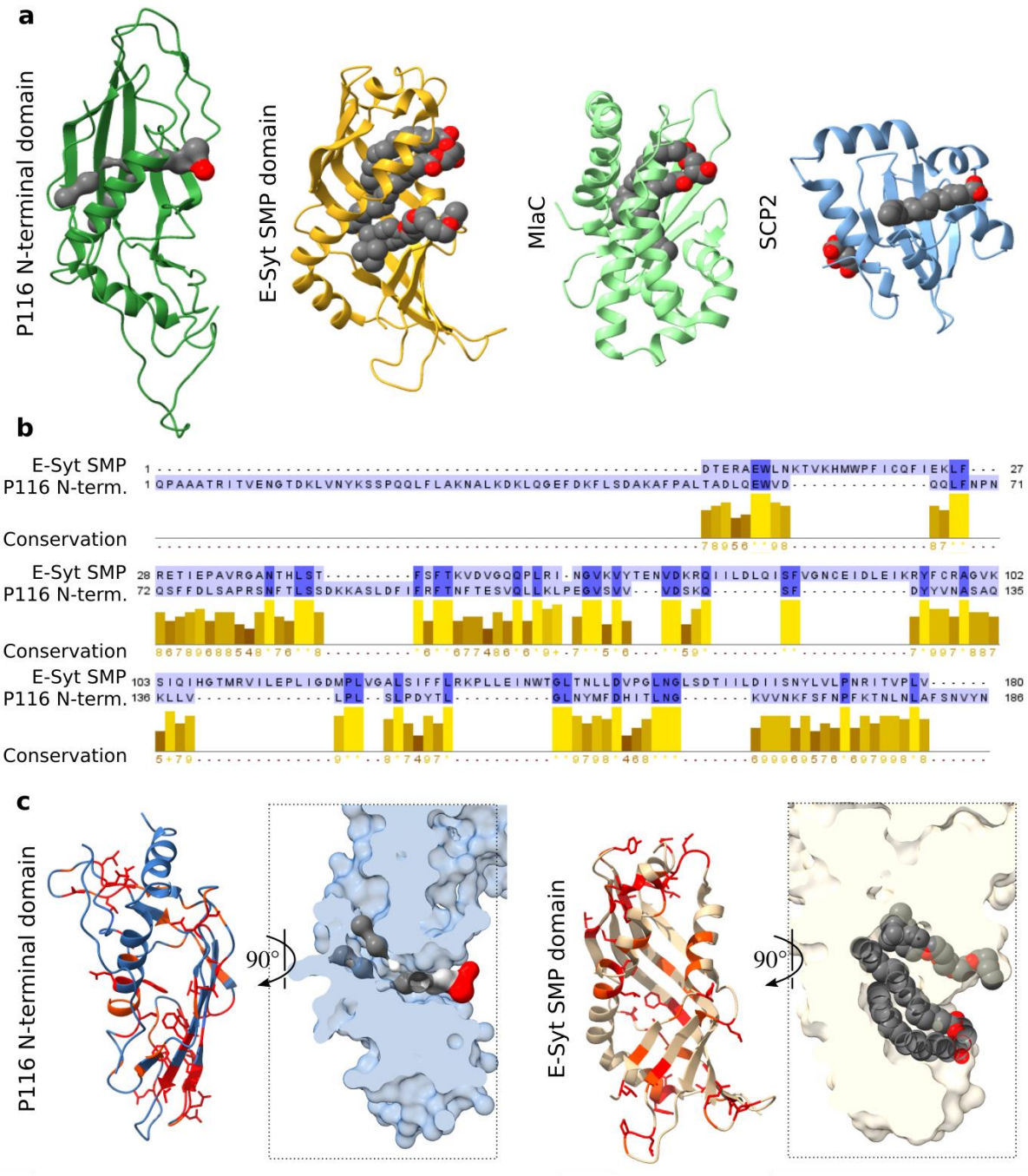
	1	2	3
	Auto-fluorescence control	Spontaneous fusion/ transfer control	Lipid transfer with P116
AVERAGE	9.55	15.45	53.33
STDEV	3.34	5.42	15.17
n	21	21	44

P (two tail)	1 vs. 2	0.000125543	***
	2 vs. 3	2.82299E-21	***
	1 vs. 3	4.53119E-24	***

P values originate from an unpaired T-test

Extended Data Table 2: Single-particle cryo-EM data collection and processing

	peptide filled P116 (30–957) **EMD-18476**	filled truncated P116 (246–818) **EMD-18477**	empty monomer of truncated P116 **EMD-18478**
Microscope	FEI Titan Krios	FEI Titan Krios	FEI Titan Krios G3i
Detector	Gatan K2 Summit	Gatan K2 Summit	Gatan K3 Summit
Acquisition Software	SerialEM 4.10beta	SerialEM 4.10beta	EPU v.3.1.3
Magnification	165,000x	165,000x	105,000x
Voltage (kV)	300	300	300
Electron exposure ($e^-/Å^2$)	50	50	50
Defocus range (μm)	-1 to -3.5	-1 to -3.5	-0.8 to -3.5
Pixel size (Å)	0.819	0.819	0.837
Symmetry imposed	C1	C1	C1
Initial particle images	3,463,490	1,197,649	6,997,649
Final particle images	1,065,351	101,747	518,561
Map Resolution (Å)	3.34	4.30	5.43
FSC threshold	0.143	0.143	0.143
Map resolution range (Å)	2.5–8	3.5–9	4–13
Number of frames	22	50	50
Micrographs used	6,007	4,314	8,002
Processing software	cryoSPARC v4.2.1	cryoSPARC v4.2.1	cryoSPARC v4.2.1
Motion correction	cryoSPARC v4.2.1	cryoSPARC v4.2.1	cryoSPARC v4.2.1
CTF estimation	cryoSPARC v4.2.1	cryoSPARC v4.2.1	cryoSPARC v4.2.1
Particle images after 2D classification	1,491,940	225,218	1,470,056
Map sharpening B factor	-120	-175	-407



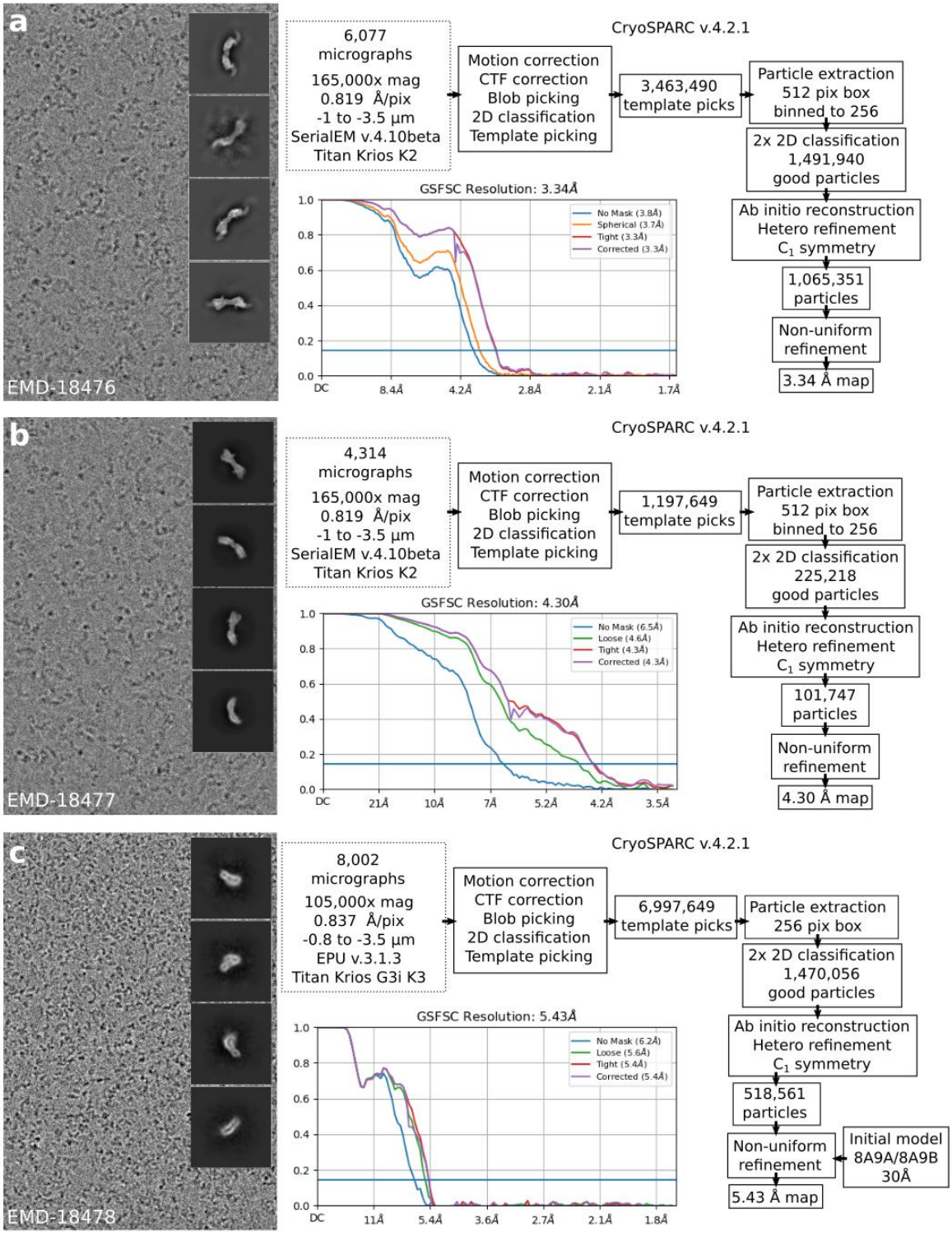
Extended Data Figure 1: Structural comparison of P116 N-terminal domain with small lipid shuttle proteins.

a, The folds of the P116 N-terminal domain (PDB: 8A9A), the E-Syt SMP domain (PDB: 4P42), MlaC (PDB: 5UWA) and SCP2 (PDB: 4JGX) share similarities: an antiparallel β-sheet covers three sides of a hydrophobic channel, while the remaining side is closed by one or more α-helices. Proteins are shown as ribbon models and lipids are shown as grey sphere models.

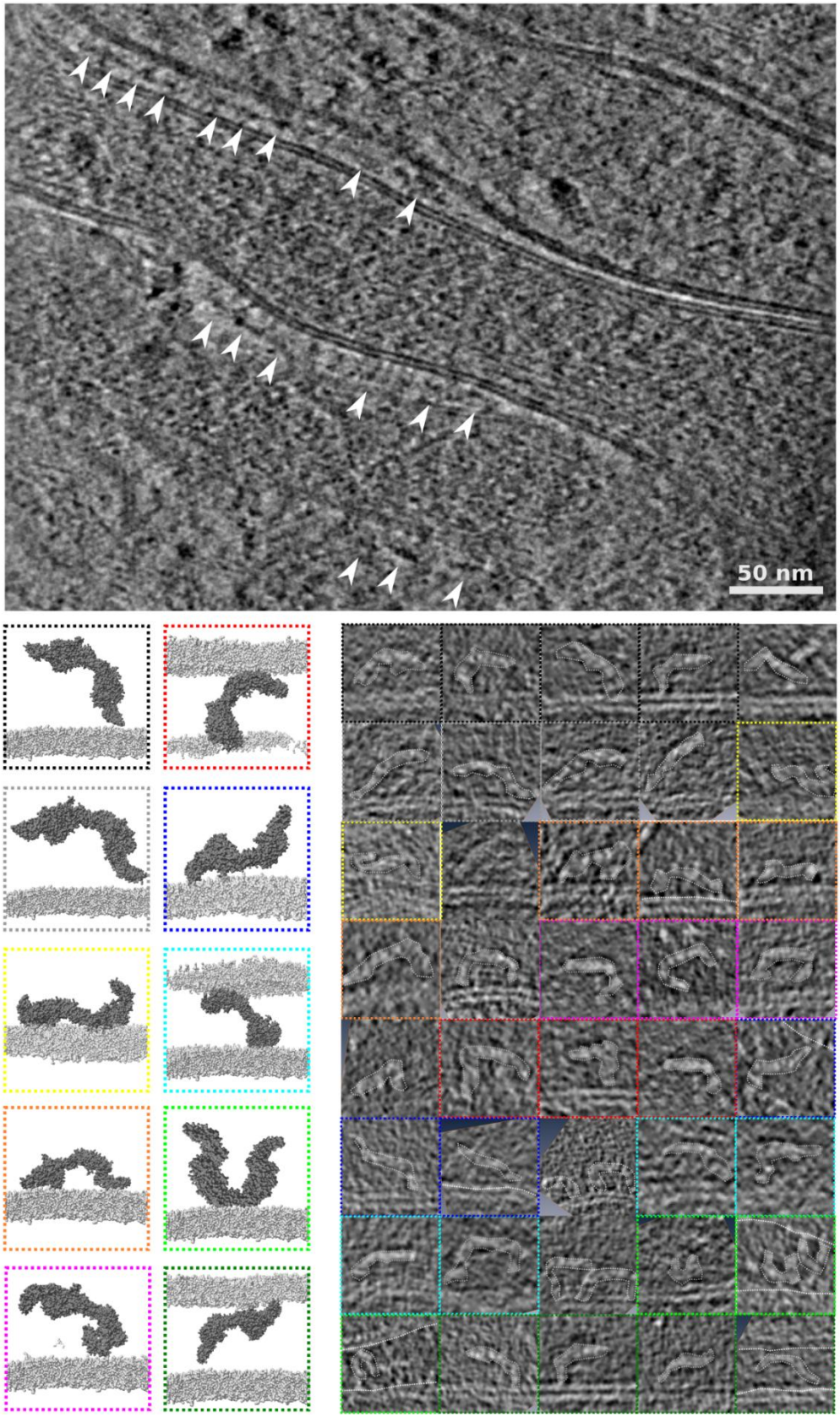
b, Sequence alignment of the P116 N-terminal domain and the E-Syt SMP domain. The sequences are colored by percentage identity. The alignment has 14% sequence identity, 25% similarity and 55% gaps.

c, Detailed structural comparison of the P116 N-terminal domain and the E-Syt SMP domain. Identical amino acids are shown in red as stick models; closely related amino

acids are shown in orange. Lipid tails are colored grey, lipid head groups are colored red.

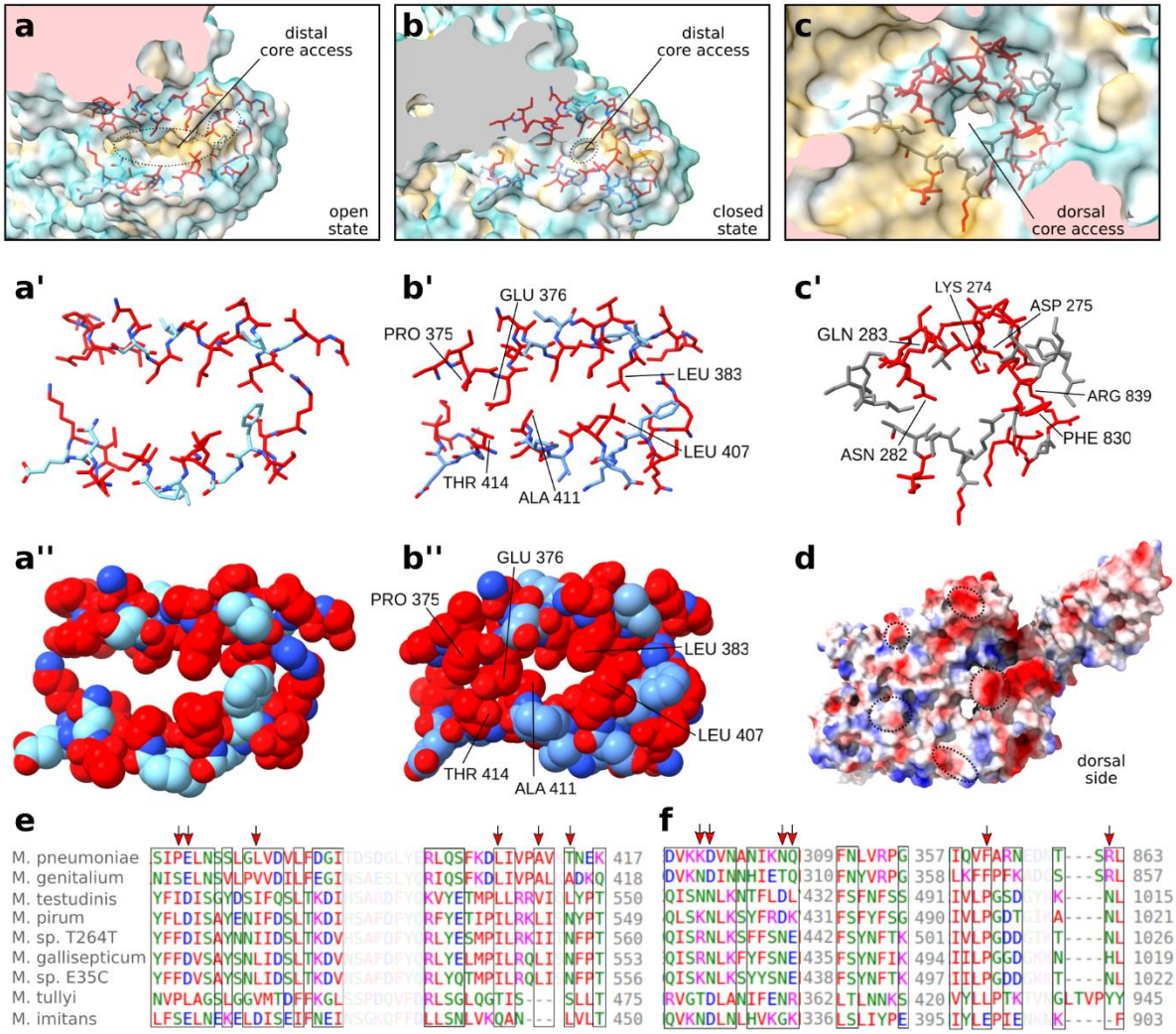


Extended Data Figure 2: Cryo-EM data acquisition and processing.
a, Peptide-filled P116 (30–957).
b, Filled P116 without the N-terminal domain (246–818).
c, Empty monomers of P116 without the N-terminal domain (246–818).



Extended Data Figure 3: Cryo-ET analysis of P116 anchored on the mycoplasma membrane. (Top) Tomographic projection slice (0.837 Å/pix) of *M. pneumoniae* cells overexpressing P116 from its native promoter. P116 is indicated by white arrows. (Bottom) Sub-tomographic projection slices of individual particles (highlighted with

dotted lines) in various conformations on the membrane, paired with conformations derived from MD simulations.



Extended Data Figure 4: Conservation of hydrophilic/hydrophobic properties of the DCA and the dorsal core access.

a/a'/a'' and b/b'/b'', DCA in filled P116 (pseudo-open state, PDB: 8A9A) and empty P116 (closed state, PDB: 8A9B).

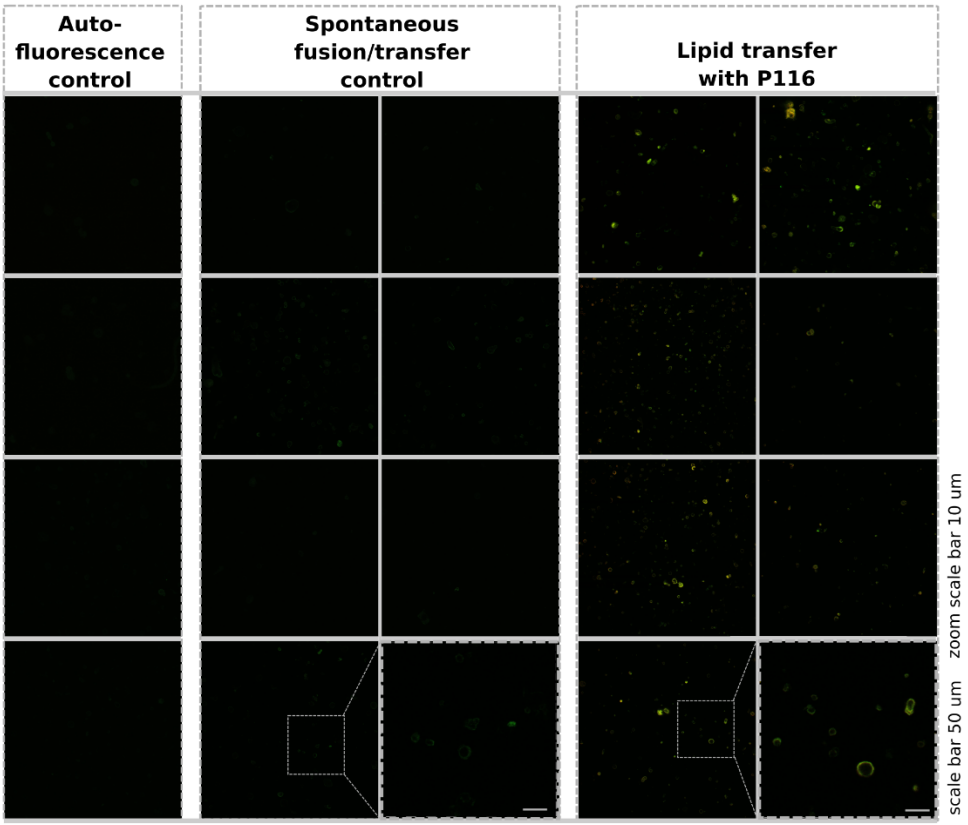
ab, The surface representations are colored by hydrophobicity factor (yellow is hydrophobic and blue is hydrophilic) with the sectioning surface in pink/grey. The position of the DCA is indicated by the dotted line.

a'/a'' and b'/b'', The amino acids building the DCA are mostly conserved in terms of their hydrophobic/hydrophilic properties (red). Non-conserved amino acids are shown in blue. The amino acids that facilitate the closing of the access in the empty state are labelled with name and number.

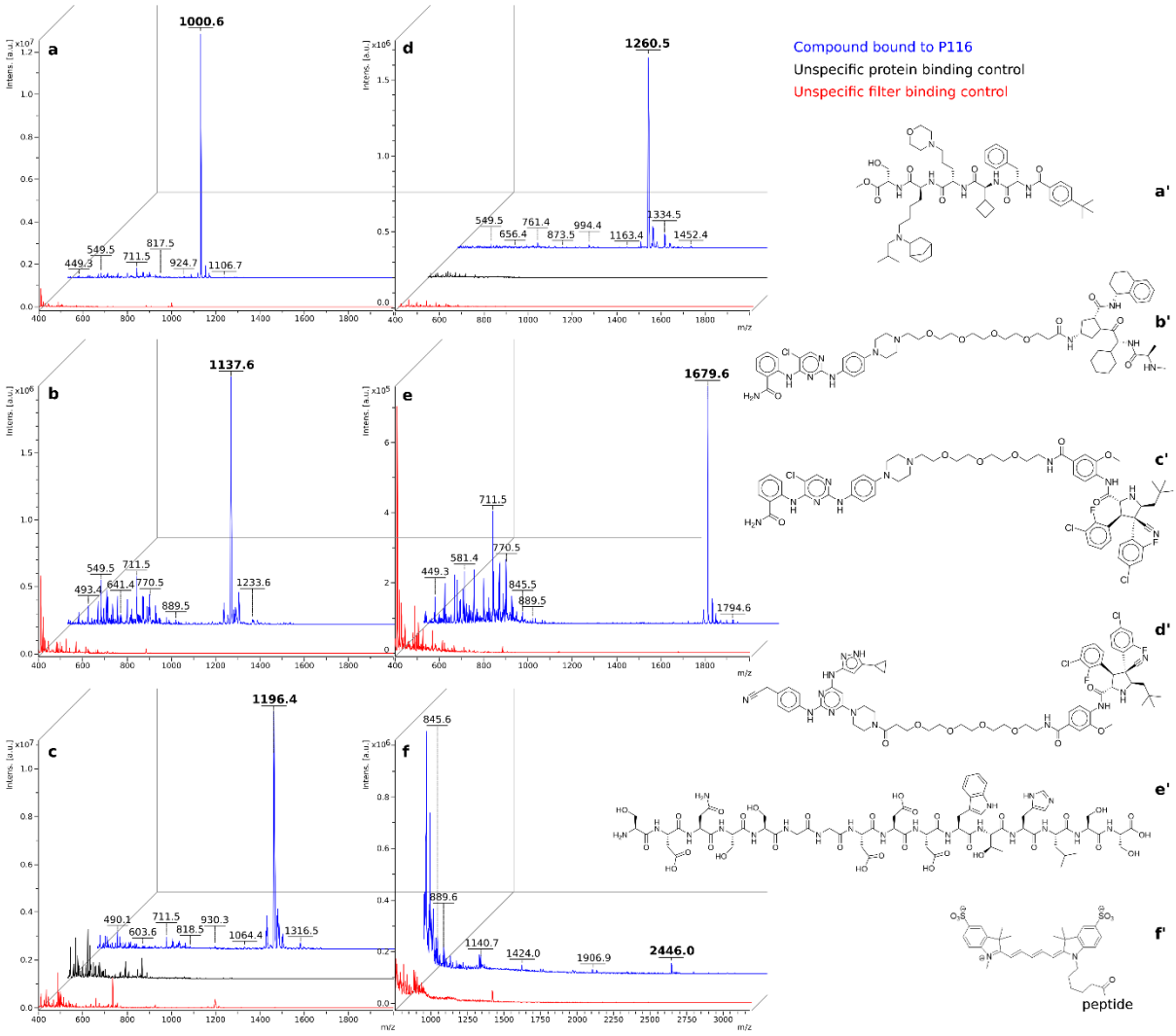
c/c', Dorsal core access in filled P116 (no difference between filled and empty P116) from the inside of the cavity. Conserved amino acids are shown in red, non-conserved amino acids in grey.

d, View of the dorsal side of P116. The surface representation is colored by electrostatic potential (acidic is red, basic is blue).

e/f, Sequence alignment of *M. pneumoniae* P116 with eight other Mycoplasma species. The areas of the sequence that comprise the distal (**e**) and dorsal (**f**) core access are shown. Amino acids that are identical or conserved in terms of their hydrophobic/hydrophilic properties are indicated by black boxes. The amino acids labelled with name and number in b'/b''/c' are indicated with red arrows.



Extended Data Figure 4: Confocal light microscopy images of delivery into DPPC liposomes. Merge of Dansyl and NBD channels. Left column: DPPC liposomes (auto-fluorescence control). Middle column: Workflow without P116 to control for spontaneous fusion/transfer control. Right column: Vesicles incubated with P116, according to the workflow shown in Figure 2. The experiment was done in triplicate. A statistical analysis of the liposome fluorescence can be found in Figure 3 and Extended Data Table 1.



Extended Data Figure 6: Analysis of hydrophobic compound binding by P116. a-f, Mass spectra of compound bound to P116 (blue), to P110 or P140 (other mycoplasma membrane proteins, black) and to filter (red).

a'–f', Structures of tested compounds. Compound f is the same as e but with an additional Cy5 fluorophore bound to its C-terminus. Peaks corresponding to each compound are annotated in bold in the respective spectrum.