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Supramolecular organization and dynamics of mannosylated phosphatidylinositol lipids in the mycobacterial plasma membrane

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1 **Supramolecular Organisation and Dynamics of**
2 **Mannosylated Phosphatidylinositol Lipids in the Mycobacterial Plasma**
3 **Membrane**

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66

67 **Abstract**

68 *Mycobacterium tuberculosis* (*Mtb*) is the causative agent of tuberculosis (TB), a
69 disease that claims ~1.6 million lives annually. The current treatment regime is long
70 and expensive, and missed doses contribute to drug resistance. Therefore,
71 development of new anti-TB drugs remains one of the highest public health priorities.
72 *Mtb* has evolved a complex cell envelope that represents a formidable barrier to
73 antibiotics. The *Mtb* cell envelop consists of four distinct layers enriched for *Mtb*
74 specific lipids and glycans. Although the outer membrane, comprised of mycolic acid
75 esters, has been extensively studied, less is known about the plasma membrane,
76 which also plays a critical role in impacting antibiotic efficacy. The *Mtb* plasma
77 membrane has a unique lipid composition, with mannosylated phosphatidylinositol
78 lipids (phosphatidyl-*myo*inositol mannosides, PIMs) comprising more than 50% of the
79 lipids. However, the role of PIMs in the structure and function of the membrane
80 remains elusive. Here, we used multiscale molecular dynamics (MD) simulations to
81 understand the structure-function relationship of the PIM lipid family and decipher how
82 they self-organize to shape the biophysical properties of mycobacterial plasma
83 membranes. We assess both symmetric and asymmetric assemblies of the *Mtb*
84 plasma membrane, and compare this with residue distributions of *Mtb* integral
85 membrane protein structures. To further validate the model, we tested known anti-TB
86 drugs and demonstrated that our models agree with experimental results. Thus, our
87 work sheds new light on the organization of the mycobacterial plasma membrane. This
88 paves the way for future studies on antibiotic development and understanding *Mtb*
89 membrane protein function.

90

91 **Significance statement**

92 Understanding the biophysical properties of mycobacterial membranes is of crucial
93 interest, especially in the context of drug discovery, but there is no realistic model of
94 mycobacterial membranes. In this work, we have developed models of inner
95 membrane of mycobacteria mainly constituted of mannosylated phosphatidylinositol
96 lipids. Our results provide significant and unexpected insights into the molecular
97 details of mycobacterial membranes. Especially, they highlight how recently
98 determined proteins can accommodate into this specific membrane and how its lipid
99 composition can affect antibiotics diffusion.

100

101 **Introduction**

102 Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (*Mtb*). In 2021 alone,
103 there were an estimated 10.6 million new *Mtb* infections, leading to 1.6 million deaths
104 (1). Thus, *Mtb* is one of the world's leading infectious killers, despite the availability of
105 both a treatment regime and vaccine. The current course of antibiotics for drug
106 susceptible TB cases can last as long as 6 months and consists of four drugs given in
107 combination (2). This is not only expensive and demanding for the patient, but also
108 encourages non-compliance that contributes towards the rise in multi-drug resistant
109 and extremely drug-resistant TB (3). It is obvious that new treatments and a better
110 vaccine are needed to meet the World Health Organization's 'End TB Strategy'. Their
111 plan aims to reduce TB-related deaths by 90% by 2030, and thereby curtail the
112 enormous public health cost caused by TB (4). The COVID-19 pandemic has undone
113 some of the progress that had been made in the treatment of TB as fewer people were
114 able to be diagnosed or access medication (5), which might result in a worldwide surge
115 of untreatable cases. Thus, there is a pressing need for innovative research into the
116 mechanisms of *Mtb* virulence and its ability to survive within the host for extended
117 periods to help develop alternative intervention strategies.

118 One issue in anti-TB drug discovery is the complexity of the mycobacterial cell
119 envelope (6, 7). This cell envelope consists of an array of lipids contributing to both
120 hydrophobic and polar regions of various thicknesses and densities, making it
121 extremely challenging to predict how molecules will cross this barrier and enter the
122 cell. The *Mtb* cell envelope has four distinct layers: the outer layer (or the
123 *mycomembrane*) comprised of mycolic acid esters and other complex lipids (such as
124 phthiocerol dimycoserolates), an arabinogalactan-peptidoglycan layer, the
125 periplasmic space and the inner membrane, or the plasma membrane (8). The
126 mycobacterial plasma membrane plays a key role in controlling nutrient/antibiotic
127 uptake and contains important membrane proteins that are targets for antitubercular
128 drugs (9), such as SQ109 that inhibits the transporter MmpL3 (10). The membrane
129 composition is also known to change during different growth stages of the bacteria (7)
130 and may modulate drug and membrane protein diffusion. Additionally, recent studies
131 showed that *Mtb* plasma membrane organization, such as the formation of functional
132 membrane microdomains (11, 12), can affect the survival ability of mycobacteria.
133 Therefore, understanding the molecular organization and dynamics of the *Mtb* plasma
134 membrane is essential for developing effective drug candidates.

135 The mycobacterial plasma membrane is composed of a variety of lipids and
136 glycolipids, the most abundant being (**Figure 1A**): cardiolipin (CL),
137 phosphatidylethanolamine (PE), phosphatidyl-*myo*inositol (PI), trehalose
138 monomycolate (TMM) and phosphatidyl-*myo*inositol mannosides (PIMs) (13), with the
139 PIMs accounting for over half the dry weight of the plasma membrane lipids (13). PIM₂
140 lipids are comprised of a modified PI core decorated with two mannose residues and
141 one acyl chain (**Figure 1B**). Furthermore, additional modifications of an acyl group
142 and up to 4 mannose sugars can be added to the core headgroup, further diversifying
143 the PIM structure (14, 15) (**Figure 1B** and **SI Figure 1**). It is still unclear if the plasma
144 membrane is symmetric or asymmetric (16). In one widely cited and accepted study,
145 it is proposed to be asymmetric (13), with Ac₂PIM₂ being the dominant species in the
146 cytoplasmic leaflet accompanied by AcPIM₂, while the periplasmic leaflet is more
147 varied, containing AcPIM₂, AcPIM₆, Ac₂PIM₆, CL, PI, PE and TMM (6, 13, 17, 18).
148 PIM₆ lipids can be further modified to LM and LAM that make up the bulk of the
149 periplasmic space (19). Additionally, lipids have been shown to modulate membrane
150 protein function (20, 21). However, due to the complexity of the mycobacterial cell
151 envelope, the dynamics and properties of the plasma membrane is extremely difficult
152 to probe experimentally.

153 Over the last decade, molecular dynamics (MD) simulations have emerged as
154 a powerful strategy for studying structural and functional aspects of biomembranes
155 (22, 23). However, at the time of writing, there are no complete models of the
156 mycobacterial plasma membrane that capture a possible asymmetry and the diverse
157 range of lipids (24). This limits our ability to understand *Mtb* plasma membrane biology,
158 model drug-lipid interactions - important for anti-TB drug discovery efforts - and
159 simulate mycobacterial membrane proteins in a native environment. Therefore, it is
160 essential to develop models of the major phospholipids of the mycobacterial plasma
161 membrane for application in MD simulations.

162 Herein, multiscale simulations were used to analyze the structure-function
163 relationship of the four main PIM lipids (AcPIM₂, Ac₂PIM₂, AcPIM₆ and Ac₂PIM₆) found
164 in the mycobacterial membrane. An asymmetric bilayer containing these lipids was
165 assembled and simulated, showing the stability of this composition. To highlight the
166 robustness of our approach, additional simulations representing (in total) two different
167 asymmetric growth stages as well as symmetric membrane configurations were
168 performed. Systematic analysis of the distribution of *Mtb* protein residues in contact

169 with lipids reveals further indications that this membrane could possibly be
170 asymmetric. We have also modelled how antibiotics, used to treat TB infections,
171 diffuse through the membrane and interact with membrane proteins and demonstrated
172 that our models agree with experimental results (25). Overall, we have developed
173 robust possible representations of the mycobacterial plasma membrane that will
174 enable studies of membrane dynamics, lipid interactions with integral membrane
175 proteins, and diffusion of antibiotics across this barrier.

176

177

178 **Methods**

179

180 *Building the coarse-grained lipid parameters*

181 The CG models of the lipids were parametrized for the newly released MARTINI 3
182 force field (26) and generated using the protocol described for small molecules (27)
183 on the MARTINI website ([http://cgmartini.nl/index.php/martini-3-](http://cgmartini.nl/index.php/martini-3-tutorials/parameterizing-a-new-small-molecule)
184 [tutorials/parameterizing-a-new-small-molecule](http://cgmartini.nl/index.php/martini-3-tutorials/parameterizing-a-new-small-molecule)). The bead types and mapping to the
185 PIM molecules were performed manually, comparing with the recently published CG
186 model of PI (28). Atoms were grouped according to functional groups, in sets of 3-5
187 non-hydrogen atoms. The CG mapping of Ac_xPIM_x is shown in **Figures 1B,C**. The
188 parameter files that describe the bonds, constraints, and angles were assembled
189 based on the previously described data (26, 28, 29) as an initial estimate.

190 Simulations of each lipid were set up using a modified version of *insane.py*
191 python script (30), embedding one copy of a PIM lipid in a 10 x 10 nm²
192 phosphatidylcholine (PC) bilayer. The system was solvated with MARTINI water (26)
193 and neutralized with 150 mM NaCl, followed by minimization using the steepest
194 descents algorithm. The system was then simulated for 3 μ s using a timestep of 20 fs
195 at 310 K. The lipids, solvent and ions were temperature coupled separately. The
196 velocity rescale (31) and Parrinello-Rahman (32) coupling methods were used with
197 the time constants $\tau_T = 1.0$ ps and $\tau_p = 12.0$ ps for temperature and pressure,
198 respectively. Simulations were run using GROMACS version 2021.3 (33). The
199 reaction-field algorithm (34) was used for electrostatics interactions with a cut-off of
200 1.1 nm. A single cut-off of 1.1 nm was used for the van der Waals interaction. Five
201 repeats were performed for each lipid (**SI Figure 2**).

202

203 *Generating atomistic data*

204 Parameters for the atomistic (AT) Ac_xPIM_x lipids were generated using the CHARMM
205 force field (35). The CHARMM-GUI (36) server was used to set up the lipid systems
206 for GROMACS with the CHARMM36m force field (37, 38). One PIM lipid was
207 embedded in an 8 x 8 nm² PC bilayer. The system was solvated with 150 mM NaCl
208 and minimized and equilibrated as per the CHARMM-GUI Membrane Builder workflow
209 (39). The system was further minimized using the steepest descents. Simulations
210 were run for 2 μs using a timestep of 2 fs at 310 K. The lipids, solvent and ions were
211 temperature coupled separately. The velocity rescale (31) and C-rescale coupling
212 methods were used with the time constants $\tau_T = 0.1$ ps and $\tau_p = 1.0$ ps for temperature
213 and pressure, respectively. Simulations were run using GROMACS version 2021.3
214 (33). The particle mesh Ewald (PME) (40) method was used for electrostatic
215 interactions with a cut-off of 1.2 nm. A single cut-off of 1.2 nm was used for the van
216 der Waals interaction. Three repeats were performed for each lipid (**SI Figure 2**).

217

218 *Refining coarse-grained parameters*

219 The AT and CG representations of the four main PIM lipids are shown in **Figure 1C**.
220 The distributions of the distances and angles were measured using the *gmx* tools
221 (*distance*, *gangle* and *analyze*). For the AT simulations, the atoms were grouped
222 according to their mapping and its center of geometry was used for calculations. The
223 values for each bond/constraint and angle were iteratively refined based on the
224 comparison of probability distribution, and the results are summarized in **SI Figures**
225 **3-8**. When there was agreement between the AT and CG data, the solvent accessible
226 surface area was measured using the *gmx sasa* tool to verify that the models behaved
227 the same (**SI Figure 8C**). Diffusion, shape of the lipids, clustering of ions, aggregation
228 and interaction with other lipids were also measured on a single lipid level (**SI Methods**
229 and **SI Figures 9-19**). As typical for MARTINI (41), dihedral terms are not defined in
230 the parameters.

231

232 *Measuring area per lipid*

233 Before setting up the complete bilayer system, the area per lipid was measured. The
234 simulations were assembled as described above for CG, using a homogenous
235 Ac_xPIM_x membrane (**SI Figure 2**). The area of XY-dimension was measured using
236 *gmx energy* and then dividing the area by the number of lipids in one leaflet over the

237 course of the trajectory, and the final values were extracted using *gmx analyze*, as per
238 the protocol described on the MARTINI website ([http://cgmartini.nl/index.php/tutorials-](http://cgmartini.nl/index.php/tutorials-general-introduction-gmx5/bilayers-gmx5#Area-per-lipid)
239 [general-introduction-gmx5/bilayers-gmx5#Area-per-lipid](http://cgmartini.nl/index.php/tutorials-general-introduction-gmx5/bilayers-gmx5#Area-per-lipid)).

240

241 *Bilayer composition and set-up*

242 The ratio of lipids in each bilayer was obtained by using their molecular weight and the
243 previously reported dry masses (13) of the individual lipids, and the exact calculations
244 can be seen in **SI Figure 20**. Since specific apolar lipids were not named, they were
245 not included in this study. TMM was not included as there is no available refined AT
246 model, the behavior of the mycolic acid is predicted to be complicated (42), and there
247 is a relatively small proportion of TMM predicted to be present in the plasma
248 membrane. The final composition of the plasma membrane is shown in **Figure 1A**.

249 The PI and PE lipids in mycobacteria are slightly different in structure to the
250 corresponding *E. coli* lipids described in the MARTINI force field (26, 28): a methyl
251 group replaces the alkene found in one of the acyl chains (**SI Figure 1**). Before
252 assembling the membrane, the parameters for these lipids were modified by adapting
253 the refined Ac₂PIM₂ acyl tail parameters and changing the existing tail. The CL
254 parameters were transferred over from the MARTINI 3 beta force field (21).

255 Simulations of the bilayer were set up using a modified version of *insane.py*
256 python script (30) using the composition shown in **Figure 1A**, where the area per lipid
257 for the periplasmic membrane was set to 0.92 nm² and the cytoplasmic leaflet set to
258 1.13 nm². The systems were then treated the same as described for the initial CG
259 systems with added equilibration steps as per the Membrane Builder workflow (39)
260 before the production simulation. There were two types of system assembled to
261 represent the asymmetric membrane previously mentioned (13). The first one had an
262 initial simulation box size of 20 x 20 x 15 nm³ and one repeat was performed for 10 μs
263 at 290 K, 300 K, 310 K, 320 K and 350 K (**Figure 2A**). The second one had an initial
264 box size of 50 x 50 x 15 nm³ and a single repeat was performed for 10 μs at 310 K
265 (**Figure 2C** and **SI Figure 2**). The number of lipids in each system is summarized in
266 **SI Figure 21**.

267 To test how the membrane properties change with differing compositions, the
268 same assembly procedure was followed to set up a symmetric membrane and an
269 'PIM-enriched' membrane (**SI Figure 22**), with one repeat for each in a simulation box
270 of 20 x 20 x 15 nm³, and another in a box of 50 x 50 x 15 nm³, both at 310 K (**SI Figure**

271 **2).** Another membrane to capture an experimentally extracted membrane was
272 simulated for 10 μs in a $20 \times 20 \times 20 \text{ nm}^3$ simulation box at 310 K. The number of lipids
273 in each system is summarized in **SI Figure 21** and their compositions in **SI Figure 22**

274

275 *Behavior of the membrane*

276 To calculate the bending rigidities, the strategy proposed by Fowler *et al.* (43) to
277 extract the bending modulus from CG simulations was followed. The membrane
278 midplane position in Monge representation was determined by extracting the
279 coordinates of all CG beads at the extremities of lipid fatty acid chains. Using a built-
280 in function of the Mathematica software package, the positions were interpolated to
281 get a smooth function before Fourier-transforming with a fast Fourier transform
282 algorithm. From the so-obtained Fourier modes, the spectral density (or power
283 spectrum) was estimated for a tensionless membrane:

$$284 \quad S(q) = L^2 k_B T \left(1/(\kappa q^4) + 1/(\sigma_p r q^2) \right)$$

285 where L^2 is the projected area in the (x, y) plane, $k_B T$ is the thermal energy and
286 $\sigma_p r \sim 0.1 \text{ J/m}^2$ is the tension associated with lipid protrusions at the nanometer scale.
287 $S(q)q^4$ was plotted as a function of q and fit it with a second order polynomial $P(q) =$
288 $a + bq^2$, from which estimates of κ (and $\sigma_p r$ if needed) were obtained (**SI Figure 23**).

289 The error bars are standard deviations provided by the fitting function in Mathematica.

290 The density of each constituent was measured using *gmx density* (**Figure 2B**).
291 Diffusion, bilayer thickness and number of neighbors was measured using LiPyphilic
292 (44) (**Figures 2D,E, SI Methods** and **SI Figures 24-27**). The xy-positions of single
293 lipids were tracked with PLUMED (45) (**Figure 2E**). Area per lipid for the membrane
294 was calculated using FATSLIM (46) (**Figure 2F**). Plots were created using Matplotlib
295 (47).

296

297 *Analysis of the residue distribution in membrane proteins*

298 All predicted protein structures from *Mtb* (taxonomy id:83332) and *E. coli* (taxonomy
299 id:83333) labelled as transmembrane on UniProt (48) were downloaded from the
300 AlphaFold database (49), totaling 729 for *Mtb* and 1,229 for *E. coli*. Alphafold was used
301 to obtain a workable dataset of structures, expanding upon the number of
302 experimentally determined *Mtb* and *E. coli* membrane protein structures available from
303 the PDB. Each of 1,958 membrane protein structures was orientated in a membrane

304 using memembed (50) with the *-n in* flag, then converted to CG using martinize2 (51)
305 and inserted into either the asymmetrical mycobacterial (see **Figure 1A**) or *E. coli*
306 membrane (75% PE, 15% PG, 10% CL) using a modified version of the *insane.py*
307 python script (30). Each system was simulated for 10 ns. The residues within 8 Å of
308 the lipids were selected and the density over the z-coordinate plotted (**Figure 3, SI**
309 **Figure 28**).

311 *Antibiotic simulations*

312 Bedaquiline (BDQ) and isoniazid (ISZ) were mapped to CG using PyCGTOOL (52)
313 following 200 ns AT simulations with parameters from CHARMM-GUI (53) (**Figure**
314 **4A**). The pK_a of the amine in BDQ is 8.91 (54), suggesting it will be protonated at
315 physiological pH. This was considered when assigning the MARTINI 3 bead type
316 (**Figure 4A**). To validate the use of the protonated state, the same simulations with a
317 neutral BDQ molecule were performed and analyzed (**SI Figures 29A-C**). This was
318 done by replacing the Q1 particle with a SC3 bead type (**Figure 4A**). In addition, the
319 LogP for these antibiotics were calculated by perturbing the antibiotic in a box
320 containing 878 MARTINI waters or 363 octanol and 32 waters. The Lennard-Jones
321 parameters for each molecule were decoupled over 11 evenly spaced λ windows, for
322 50 ns per window. Simulations were run with 20 fs time steps in the NPT ensemble
323 with the V-rescale thermostat at 323 K and an isotropic Parrinello-Rahman pressure
324 coupling. Free energies were then computed using *gmx bar* and converted to
325 water/octanol partitioning constants. The values were then compared to data from the
326 ALOGPS online server (55). Alongside this, *gmx sasa* was used to calculate the
327 solvent accessible surface area for the antibiotics in both CG and AT. The calculations
328 were performed with 4800 dots per sphere and a probe size of 0.4 nm. Modified van
329 der Waals radii for CG beads of 0.264, 0.230, and 0.191 nm for R, S and T beads
330 were used respectively. For the Connolly surface generation, the calculations used
331 240 dots and a probe of 0.4 nm (**SI Figure 30**).

332 BDQ was first simulated with the mycobacterial membrane alone to confirm
333 association with the membrane (**SI Figures 2,31**). The *Mtb* a- and c-subunits of F-
334 ATPase, BDQ's target in the mycobacterial membrane, was modelled using
335 SwissModel (56) based on a structure from *Mycobacterium smegmatis* (25) (PDB:
336 7JGC, **Figure 4C** and **SI Figures 32A,B**). The sequence identity between *Mtb* and *M.*
337 *smegmatis* for the F-ATPase was calculated using Clustal Omega (57) at 80% and

338 72% for c- and a-subunits, respectively. The system with the protein in the asymmetric
339 mycobacterial membrane (see **Figure 1A**) was assembled using martinize2 (51),
340 memembed (50) and the modified *insane.py* python script (30), with eight molecules
341 of BDQ placed either in the periplasmic or cytoplasmic leaflet with five repeats in each
342 membrane (**SI Figure 2**). Simulations were run for 10 μ s using the same setting as
343 described above. The xyz-positions of BDQ, the lipids and the backbone beads were
344 tracked over the course of the simulations with PLUMED (45) and plotted with
345 Matplotlib (47), and the results are shown in **Figure 4D** and **SI Figures 32C,D, 33**.
346 The density of each constituent was measured using *gmx density* and plotted using
347 Matplotlib (47) and the results are shown in **Figure 4E** (with representative positions
348 shown in **Figure 4F**). The interaction of BDQ with the protein was calculated using
349 PyLipID (58) (**Figure 4G** and **SI Figures 34,35**). The same simulation set up was
350 performed with a model *E. coli* membrane (75% PE, 15% PG, 10% CL) and with the
351 symmetric mycobacterial membrane (**SI Figure 22A**). The z-position of BDQ is shown
352 in **SI Figure 32** and **SI Figures 36,37**.

353

354 *PMF calculations*

355 The potential of mean force (PMF) calculations for the F-ATPase-BDQ interaction
356 were performed (59). First, a representative pose of BDQ bound to the protein was
357 produced using PyLipID from equilibrium simulations and built into a 12 x 20 x 11 nm³
358 asymmetric mycobacterial membrane (see **Figure 1A**) membrane and minimized and
359 equilibrated, as described above. Light (50 kJ/mol/nm²) xy positional restraints were
360 added to Ala 66 on three c-subunits to prevent the protein from rotating in the
361 membrane. Following 50 ns of equilibration, the BDQ was steered away from the
362 protein along the y axis at a rate of 1 nm/ns with a 1,000 kJ/mol/nm² restraint potential.
363 Frames were extracted at 0.1 nm spacing along this coordinate to seed a total of 58 x
364 1.5 μ s production simulations with a static 1,000 kJ/mol/nm² umbrella potential
365 imposed to keep the system in the same position along the reaction coordinate. The
366 PMF mdp input files can be downloaded at <https://github.com/chelsea-brown/PIM-lipids/tree/main/PMF-files>. The PMF profiles were then constructed using the weighted
367 histogram analysis method in GROMACS (*gmx wham* (60, 61)), and employing 200
368 rounds of Bayesian bootstrapping to report statistical accuracy (**Figure 4H**).

370 For the membrane crossing PMFs, BDQ or ISZ was placed free in the solvent
371 phase, 7 nm away from the membrane periphery in either an asymmetric

372 mycobacterial membrane (see **Figure 1A**) or *E. coli* membrane. The drug was then
373 steered towards and through the membrane and into the solvent phase on the other
374 side. Windows were extracted, simulated (1 μ s for ISZ per window and 2 μ s per
375 window for BDQ) and analyzed as described above (**Figure 4B** and **SI Figure 29D** for
376 BDQ neutral). The -cycl option was imposed when running *gmx wham*.

377

378 **Results**

379

380 *PIM properties*

381 As an initial step towards developing an *in-silico* model of the mycobacterial plasma
382 membrane, we first examined lipid properties in our CG and AT models. As shown in
383 **SI Figures 3-8**, the probability distributions of bonds and angles from the CG and AT
384 simulations align well, which illustrates that the behavior of the acyl chains is similar
385 to other phospholipids (30). The Ac_xPIM_x aggregation in the AT and CG simulations is
386 comparable and shows no permanent clustering. When lipids did come into contact,
387 all areas of the molecule appear to play an equal role in the interactions (**SI Figures**
388 **15,17**). The interactions were not dominated by the sugars, as was seen with the
389 previous iteration of the MARTINI 2 force field (62), and the phospholipids present in
390 the mycobacterial membrane did not have strong interactions with the PIM lipids (**SI**
391 **Figure 18,19**). The PIM lipids show a higher affinity for ions than PC in CG simulations,
392 but the effect is less apparent in AT simulations (**SI Figure 14**). While the ion
393 concentrations were the same, the number of ions in the simulation box were different
394 (an order of magnitude higher for CG), which could explain these results. Overall, CG
395 simulations of these lipids behave similarly to AT models (interactions with ions as a
396 minor exception), therefore opening the door to significantly longer simulations by
397 decreasing the degrees of freedom in the system.

398 The area per lipid in CG for each species in a PC bilayer was found to be 0.93
399 nm² (AcPIM₂), 1.15 nm² (Ac₂PIM₂), 1.01 nm² (AcPIM₆), 1.14 nm² (Ac₂PIM₆), 0.6 nm²
400 (PI), 0.55 nm² (PE) and 1.25 nm² (CL). The diffusion coefficients for each PIM species
401 in a PC bilayer at 310 K are as follows: 6.7 x 10⁻⁷ cm²/s (AcPIM₂), 1.1 x 10⁻⁷ cm²/s
402 (Ac₂PIM₂), 7.6 x 10⁻⁷ cm²/s (AcPIM₆), 6.5 x 10⁻⁷ cm²/s (Ac₂PIM₆) (**SI Figure 9**). The
403 difference between AcPIM_x and Ac₂PIM_x shows the effect of the extra acyl tail in terms
404 of how freely these lipids diffuse through the membrane.

405 The lipid shapes are approximately the same from AT to CG (**SI Figures 10-**
406 **13**), which agrees with the comparison of the surface areas (**SI Figure 8C**) and the
407 sugar-phosphate z-distances in Ac_xPIM₆ (**SI Figures 5C,7C**). The additional mannose
408 moieties project upwards away from the membrane in both sets of simulations.
409 Interestingly, the lipid tail region is measured to occupy approximately the same
410 amount of space with three or four acyl chains. This is likely due to the placement of
411 the fourth tail, projecting downwards from the inositol sugar (as highlighted in red in
412 **Figure 1B**) and hence inhabiting space close to the other acyl chains.

413 Taken together, the lipid properties in our CG model broadly replicate those
414 seen with AT resolution. This provided the confidence to assemble these lipids into a
415 complex bilayer.

416

417 *Mycobacterial membrane biophysical properties*

418 Traditional MD simulations of bacterial membranes use symmetric bilayer
419 representation. However, a study has shown that the mycobacterial plasma
420 membrane could be an asymmetric bilayer, and we used the published composition
421 of the mycobacterial plasma membrane (13) (**Figure 1A**) (excluding apolar lipids and
422 TMM) to assemble an asymmetric bilayer (**Figures 2A,C**). This bilayer was found to
423 be stable during 10 μs simulation (**SI Movie 1**), and in good agreement with the
424 literature (8, 13). The thickness of the plasma membrane in our simulations was at 5.3
425 ± 0.1 nm (**Figure 2B**), which is slightly narrower than values obtained from imaging
426 studies (6.3 nm for *Mycobacterium bovis* and 7 nm for *Mycobacterium smegmatis*)
427 (63-65). However, we did observe thicknesses of up to 7 nm in the density plot (**Figure**
428 **2B**). *In vitro* culture conditions or growth stages of the bacteria (65) impact the
429 thickness on the membrane and could explain any minor differences with our model.

430 To test the phase behavior of this membrane, we performed simulations at
431 various temperatures ranging from 290 K to 350 K. For the whole range of
432 temperatures, lipids were in the liquid phase and allowed to diffuse freely (**Figure 2E**
433 and **SI Figure 24**). The calculated diffusion coefficients for each species in the
434 periplasmic leaflet at 310 K were as follows: 9.9 × 10⁻⁸ cm²/s (AcPIM₂), 6.9 × 10⁻⁸ cm²/s
435 (AcPIM₆), 1 × 10⁻⁷ cm²/s (Ac₂PIM₆), 1.3 × 10⁻⁷ cm²/s (PI), 1.5 × 10⁻⁷ cm²/s (PE) and 1.2
436 × 10⁻⁷ cm²/s (CL). In the cytoplasmic leaflet, the values were 6.1 × 10⁻⁸ cm²/s (AcPIM₂)
437 and 8.1 × 10⁻⁸ cm²/s (Ac₂PIM₂). Compared to isolated PIMs in a PC membrane, the
438 diffusion in this plasma membrane was roughly one order of magnitude slower.

439 AcPIM₂, which is the only lipid in both leaflets, diffused ~40% slower in the cytoplasmic
440 membrane compared with the periplasmic leaflet. Using a previously reported model
441 of mammalian plasma membrane (22), the diffusion coefficients of PE and PI were
442 calculated to be $3.3 \times 10^{-7} \text{ cm}^2/\text{s}$ and $2.8 \times 10^{-7} \text{ cm}^2/\text{s}$, showing that the diffusion in the
443 mammalian membrane is equivalent to that in the mycobacterial plasma membrane.
444 The membrane stiffness of our mycobacterial membrane was significantly lower at
445 $\kappa = 8.2 \text{ k}_B\text{T}$ compared to $13.9 \text{ k}_B\text{T}$ and $19.1 \text{ k}_B\text{T}$ for the mammalian and PC membrane
446 models, respectively (**SI Figure 23**), highlighting a unique dynamic behavior for the
447 mycobacterial plasma membrane.

448 Lipid clustering was moderate and membrane composition remained
449 heterogenous over the course of each $10 \mu\text{s}$ simulation (**Figure 2C**). This can also be
450 seen in **Figure 2D** where the number of surrounding lipids of the same type for each
451 species in the periplasmic membrane was roughly equivalent to that of any other lipid
452 species. The distribution of the area per lipid in the membrane also suggests high
453 heterogeneity (**Figure 2F**). The average area per lipid in the membrane over the
454 course of the simulation was 0.89 nm^2 and 1.18 nm^2 for the periplasmic and
455 cytoplasmic leaflets, respectively. Thus, the cytoplasmic leaflet appears to be a little
456 denser than the periplasmic leaflet. This can be related to the packing of the four acyl
457 chains of Ac₂PIM₂ lipids present in high concentration in this leaflet (66). A movie for
458 the change in area per lipid for each leaflet over the course of the simulation can be
459 found in **SI Movies 2,3**.

460 As was seen for the individual lipids in CG the overall bilayer attracted ions;
461 both Cl⁻ and Na⁺ concentration was much higher close to the bilayer, especially around
462 the sugar head groups compared to bulk solution (**Figure 2B**). It has been shown that
463 lipid-ion interactions can affect the biophysical properties of the membrane, such as
464 fluidity and stiffness, as well as the structure, which could modify the interaction with
465 proteins (67).

466 The mycobacterial plasma membrane has been reported to change at different
467 growth stages (7, 68), but the study that originally proposed the composition was
468 based solely on late-exponential-phase cells. To explore a wider range of possible
469 membrane compositions, we designed an 'PIM-enriched' bilayer, where the amount
470 of PIM lipids in the periplasmic leaflet was increased to reflect cells in rapid growth (7)
471 (see compositions in **SI Figure 22**). In this case, the overall biophysical properties of
472 that leaflet were very similar to those seen in **Figure 2**, but interestingly a change in

473 the diffusion and density of the cytoplasmic leaflet was observed (**SI Figures 26,27**).
474 Increasing the proportion of lipids in the periplasmic leaflet that have 3 or 4 acyl chains
475 could mean that the lipid tails make more inter-leaflet interactions and hence slow
476 down diffusion and make the leaflet denser. This decrease in fluidity in the membrane
477 could impact antibiotic diffusion through the membrane (13, 69) which would make the
478 bacteria less susceptible to treatment at a time when it is most vulnerable.

479 We have also tested alternative plasma membrane compositions with a
480 symmetrical configuration. First, we performed a model of a membrane recapitulating
481 the composition being experimentally derived using a combination of mass
482 spectrometry and NMR (see composition in **SI Figures 22,38**). This symmetrical
483 configuration is stable, and the dynamics of PIM lipids in CG-MD simulations agreed
484 with solid state NMR results (see **SI Figures 38,39**). This data shows that the acyl
485 chains extremities are highly mobile, while the core of the acyl chains displayed a more
486 rigid structure. Interestingly, the sugar moieties adopted an intermediate dynamic
487 regime, as seen from experimental NMR results (**SI Figure 38E**) and MD simulations
488 (**SI Figure 39B**). We then designed a model of symmetrical membrane system with a
489 lipid composition that mimics *Mtb* membranes (see composition in **SI Figure 22A**).
490 This membrane showed similar interactions of lipids compared to the asymmetric
491 composition and also similar rates of diffusion (**SI Figures 25,27**). Clustering of ions
492 around the sugar headgroups was still observed.

493 Taken together our observations suggest that PIM lipids play a critical role in
494 the dynamic and structuration of mycobacterial plasma membrane. Both the
495 asymmetric and symmetric configurations of the membrane were equally stable and
496 our analyses show that both share similar dynamic properties. This work demonstrates
497 the plausible arrangements of the plasma membrane and provides a mechanism to
498 easily study new membrane compositions based on future experimental results.

499

500 *Asymmetrical distribution of residues at the surface of Mtb membrane proteins*

501 The lipids of the plasma membrane do not exist in isolation, but rather in cooperation
502 with membrane proteins. These proteins could provide insights into how this
503 membrane exists natively (70). To explore this, we used the accurate, fast and reliable
504 workflows of AlphaFold (49) and memembed (50), to insert all transmembrane
505 proteins from *Mtb* and *E. coli* into their native-like membranes (**Figure 3A,B**). We then
506 assessed the distribution of residues in proximity to the lipid bilayer (**Figure 3C**).

507 For *E. coli*, the distribution is as expected and similar to results previously
508 shown (21). The asymmetry of Arg and Lys reflect the 'positive inside rule' (71-73)
509 present in bacteria which aids in the correct insertion of the transmembrane helices.
510 The symmetric nature of the lipid bilayer is otherwise reflected in the distribution of the
511 residues (**Figure 3C**, **SI Figure 28**). The *Mtb* distribution of Arg also shows a strong
512 'positive inside rule'. This is at a higher intensity than in *E. coli*, likely due to the low
513 levels of Lys observed (**Figure 3C**). Furthermore, the decrease in the bulky Phe and
514 Tyr residues (**Figure 3C**) in the cytoplasmic leaflet compared to the periplasmic leaflet
515 agrees with the increased density seen in an asymmetric membrane (**Figure 2B** and
516 **SI Figure 26B**).

517 These results add evidence to an asymmetric model previously proposed (13).
518 In addition to validating the model proposed in this work, it could aid understanding of
519 how mycobacterial membrane proteins differ from those found in other organisms and
520 hence aid targeted therapies.

521

522 *Asymmetric interaction of the membrane with antibiotics*

523 We were interested in testing how the organization of this membrane affects the
524 behavior of other molecules, such as antibiotics. We tested two widely used anti-TB
525 antibiotics: Isoniazid (ISZ) and Bedaquiline (BDQ) (**Figure 4A**). To validate the
526 behavior of these small molecules as well as the bond lengths/angles being account
527 for through PyCGTOOL (52) (**SI Figure 30**), the LogP and solvent accessible surface
528 area were calculated for each and compared to predicted values and atomistic values,
529 respectively. The values for LogP for neutral BDQ (5.92 for CG, 6.37 predicted) and
530 ISZ (-1.10 for CG, -0.71 predicted) agree very well, as do the solvent accessible
531 surface area values (BDQ values $13.01 \pm 0.49 \text{ nm}^2$ for CG and 13.69 ± 1.11 for AT;
532 ISZ values $7.48 \pm 0.14 \text{ nm}^2$ for CG and 7.35 ± 0.04 for AT).

533 ISZ is a first line anti-TB treatment that targets InhA, a cytosolic enzyme that is
534 essential for mycolic acid synthesis (74). BDQ on the other hand is a last line antibiotic
535 that targets the membrane protein complex of the ATP synthase (75, 76). The PMF
536 results for ISZ and BDQ show that the mycobacterial membrane behaves as expected
537 regarding the passage of a small molecule through a lipid rich membrane, showing
538 favorable interactions with the largely hydrophobic BDQ and unfavourable interactions
539 with hydrophilic ISZ (**Figure 4B**). The positive charge present on BDQ could account
540 for the sharp free energy barrier at the membrane midplane. To assess the effect of

541 the charge on the passage of BDQ since the protonation state is unknown, we
542 modeled a neutral BDQ where the charged Q1 bead (see **Figure 4A**) was replaced
543 by SC3. The PMF results with the neutral BDQ show a reduction in the energy barrier
544 at this point (**SI Figure 29D**). We note that the passage of these drugs through an *E.*
545 *coli* inner membrane is symmetric from the mid-plane of the membrane as expected.
546 The interactions of BDQ with the periplasmic leaflet of the mycobacterial membrane
547 were ~6 kJ/mol stronger than for the simple membrane and the mycobacterial
548 cytoplasmic leaflet. This is possibly due to the presence of tetraacylated PIMs in the
549 mycobacterial periplasmic leaflet. Roughly the same difference between the
550 mycobacterial and simple membranes was observed for ISZ at the mid-membrane
551 region, showing less favorable passage through the membrane. These results suggest
552 that ISZ, a first-line TB drug, is unlikely to freely diffuse through the membrane, which
553 is in contrast with the previously proposed uptake mechanism based on passive
554 diffusion (77-79). Small differences were seen for ISZ interactions at the interfaces of
555 the mycobacterial and simple membrane periplasmic membrane. This could be due to
556 the presence of certain PIM lipids in this leaflet. In our simulations, when the BDQ
557 starting position was in the bulk solvent, the drug quickly associated with the
558 membrane, as shown in **SI Figure 31**, and displayed no strong preference for either
559 leaflet in these simulations.

560 BDQ has been shown to target the mycobacterial ATP synthase (Rv1304-
561 Rv1311), a membrane protein complex. A cryo-EM structure from *M. smegmatis*
562 (PDB: 7JGC) shows multiple binding sites for BDQ at the interface of the c-subunits
563 and the interface between the a- and c- subunits (25), where a highly negatively
564 charged area is located (**SI Figure 32A,B**). Using the available cryo-EM data, we
565 performed 10 simulations of 10 μ s each to examine how *Mtb* ATP synthase complex
566 behaves in our asymmetric mycobacterial plasma membrane model. ATP synthase
567 was stable in the membrane and that there were no significant perturbations of the
568 bilayer by the protein. The lipids of the mycobacterial membrane did not show any
569 strong interactions with the protein (**Figure 4D** and **SI Figure 33**), apart from CL that
570 localizes in the a-subunit and around the c-ring (**Figure 4D**) in positions similar to
571 those observed in a previous study (80). The exact values for CL occupancy of each
572 residue are shown in **SI Figure 35A**. Minimal interactions between BDQ and the
573 ATPase occurred when the drug started in the periplasmic leaflet; however, when BDQ
574 was introduced in the cytoplasmic leaflet, significant occupation of the binding sites on

575 ATPase were observed (**Figures 4D-G** and **SI Figures 32C,D**). The interactions were
576 reduced with the neutral BDQ model (**SI Figures 29A-C**). Of note, no such leaflet
577 differences were observed when we tested the *E. coli* symmetric membrane model (**SI**
578 **Figures 36C,D; SI Figure 37**), but with a symmetric mycobacterial membrane (**SI**
579 **Figure 32**). This suggests that the lipid composition plays a crucial role in the
580 regulation of antibiotic interactions.

581 Furthermore, the interactions of the charged BDQ we observed matched well
582 with what was previously seen in the cryo-EM structure (25), with an occupancy of
583 ~30% of the simulation time on some subunits (**Figure 4G**). The occupancy value
584 averaged over all subunits is shown in **SI Figure 35B**. The simulations were able to
585 identify all three types of sites reported previously (25). Most interactions were seen
586 through the leading site (46% of the time, $K_{off} = 5.2 \mu s^{-1}$) followed by the lagging site
587 (22% of the time, $K_{off} = 7.6 \mu s^{-1}$) and finally further interactions around the rotor (36%
588 of the time, $K_{off} = 1.2 \mu s^{-1}$, **SI Figure 34**). For the leading site, the PMF calculations
589 confirm this as a binding site, giving a moderate energy well of approximately 7 kJ/mol
590 (**Figure 4H**) and making it equivalent to a typical cholesterol-protein binding interaction
591 (81). Tracking the z-position of the central bead from the antibiotic over the course of
592 the unbiased simulations with the protein shows that the binding sites are occupied to
593 some extent by each of the drug molecules and some are flipped to the outer leaflet
594 from the plasma leaflet, as shown in **SI Figure 32C**.

595 These studies illustrate that our asymmetrical mycobacterial plasma
596 membrane model can be combined with studies of antibiotics, as well as with studies
597 of membrane proteins and their interactions with lipids and/or antibiotics. Taken
598 together, our simulations suggest that BDQ must enter the target protein from the
599 cytoplasmic side, which agrees with resistance mechanisms against BDQ that involve
600 upregulation of MmpL5, a drug efflux pump (82, 83). Importantly, an *E. coli* membrane
601 could not capture this feature, highlighting that the mycobacterial membrane model is
602 a more accurate and functionally relevant representation of the *Mtb* plasma
603 membrane.

604

605 **Discussion**

606 Here, we provide models for lipids constituting the mycobacterial plasma membrane
607 focused on the PIM lipids, as they represent more than 50% of the total lipid content
608 in *Mtb*. Thus, understanding how these lipids behave on an individual level and as a

609 constituent of a membrane could provide key insight into the intrinsic resistance of *Mtb*
610 to antibiotics.

611 In this CG model, clustering of ions around these lipids was observed at both
612 the single lipid and bilayer level, probing how this affects the biophysical properties of
613 the bilayer and whether this could be exploited for treatment of TB is an interesting
614 area for future research. The lipids did not cluster together excessively over the
615 timescales studied and all diffused well through the membrane. This confirmed that
616 the mycobacterial cell envelope is dynamic, which could potentially be an important
617 insight into how this cell wall functions. A low membrane bending rigidity compared to
618 a PC and eukaryotic plasma membrane is interesting and could suggest the
619 importance of other cell envelope components in maintaining the shape of the cell.

620 The simulations confirmed that our asymmetric plasma membrane model is
621 stable with a composition of over 50% PIM lipids. However, considering that this
622 arrangement has not been experimentally validated, a symmetric and 'enriched PIM'
623 asymmetric membrane was also shown to share similar biophysical properties. The
624 high proportion of PIM lipids resulted in some unique properties, most notably lower
625 membrane stiffness, which may have important implications for membrane
626 microdomain formation, drug penetration, and integral membrane protein behavior. In
627 this context, the integral membrane proteins from *Mtb* are of interest for the
628 development of new antibiotics for TB (9), and we show evidence that our membrane
629 model can be used to simulate proteins in a native lipid environment to determine any
630 key lipid interactions, as illustrated by our study of antibiotic BDQ and its target,
631 ATPase. In addition, an overall comparison of plasma membrane protein residues
632 found in contact with the lipid bilayer revealed differences between *Mtb* and *E. coli*,
633 supporting the asymmetric nature of the mycobacterial plasma membrane model. This
634 potentially hints at topological differences between proteins in mycobacteria and those
635 within other bacteria. If this is the case, currently unknown proteins which maintain the
636 asymmetry might be present (such as those identified in animal cells (84)), and would
637 constitute interesting drug targets to explore.

638 Potential limitations of our model stem from the reduced type of lipids we
639 included in this initial development. Thus, future studies will focus on expanding the
640 model to include apolar lipids (such as triglycerides (17)), TMM and LM/LAM.
641 Moreover, future simulations with proteins and probing protein/lipid interactions with
642 atomistic resolution could help further elucidate the role of these complex lipids.

643 In conclusion, our study is a starting point for building an entire mycobacterial
644 cell envelope, as done for gram-negative bacteria (85). Other mycobacterial lipids
645 have already been parameterized (86), and combining them with the model described
646 here has the potential to significantly advance the field that has been lacking
647 experimental strategies for plasma membrane investigations. Additionally, the
648 mycobacterial cell envelope is known to change at different growth stages during its
649 lifecycle (7, 68, 87, 88) and in response to different environments (69). With our model
650 and set-up methods, as shown with the multiple compositions simulated, it will be
651 possible to understand drug permeability or protein behavior at different stages of
652 infection. Taken together, integrating our mycobacterial plasma membrane model into
653 broader studies of this pathogen, its pathogen/host interactions, as well as into anti-
654 TB drug discovery and development has a potential to reveal new functional insights
655 and yield improved therapies.

656

657 Data availability: <https://github.com/pstansfeld/PIM-lipids>

658 Atomistic systems and CG-membrane set-up can be performed using respectively
659 CHARMM-GUI bilayer builder (<https://charmm-gui.org/input/membrane.bilayer>) and
660 CHARMM-GUI MARTINI bilayer Maker (
661 <https://charmm-gui.org/?doc=input/martini.bilayer>)

662

663 **Author contributions**

664 C.M.B., R.A.C., W.I., P.J.S. and M.C. designed research; C.M.B., R.A.C., Y.G., Y.K.C.
665 and E.L. performed computational research; C.M.B., R.A.C. and N.D. analyzed
666 computational data; A.G., M.G., J.N. and A.L. performed experimental research and
667 analysis; C.M.B., R.A.C., A.L., P.J.S. and M.C. wrote the paper; J.N., A.L., E.F., W.I.,
668 P.J.S. and M.C. obtained funding.

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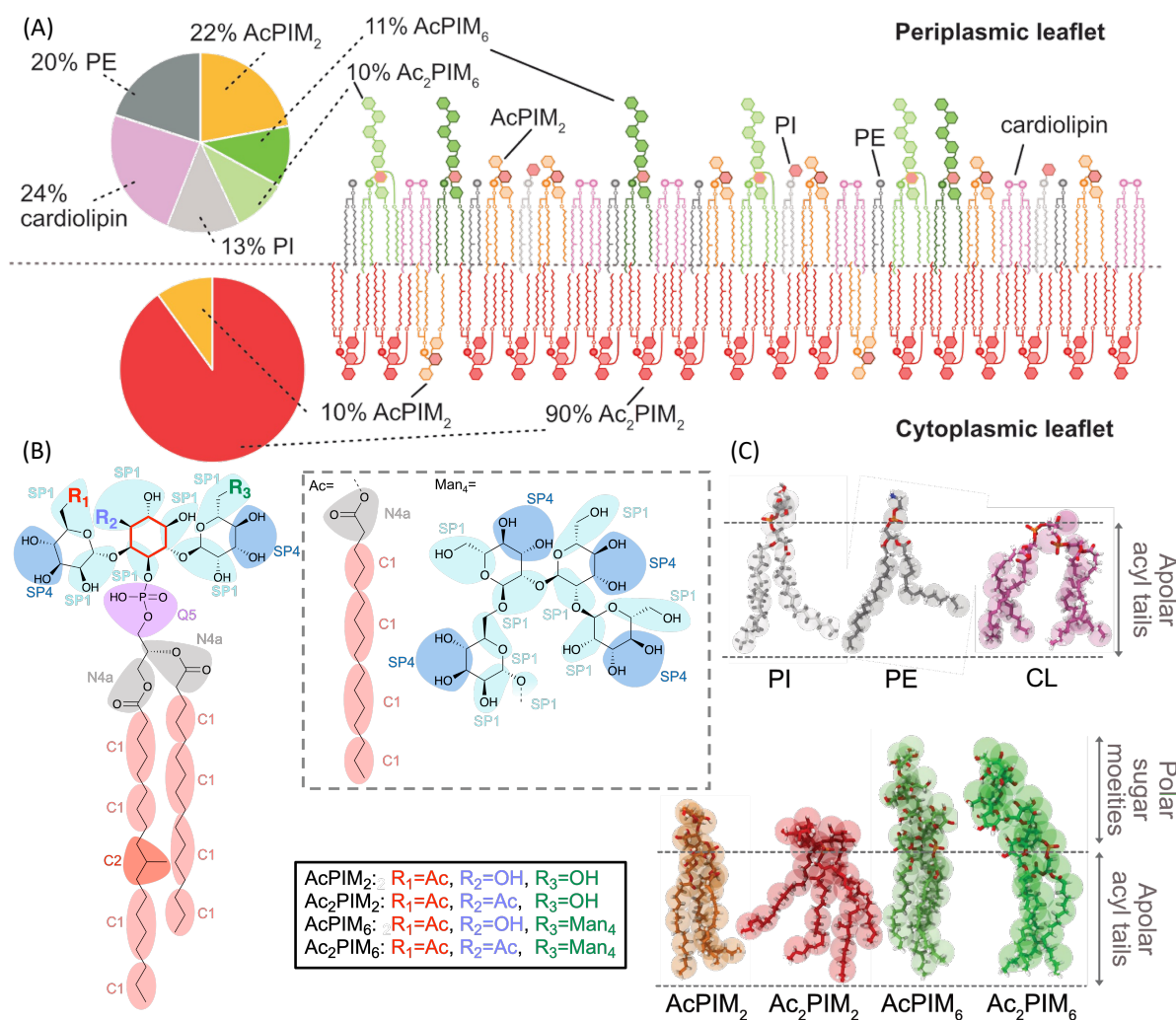
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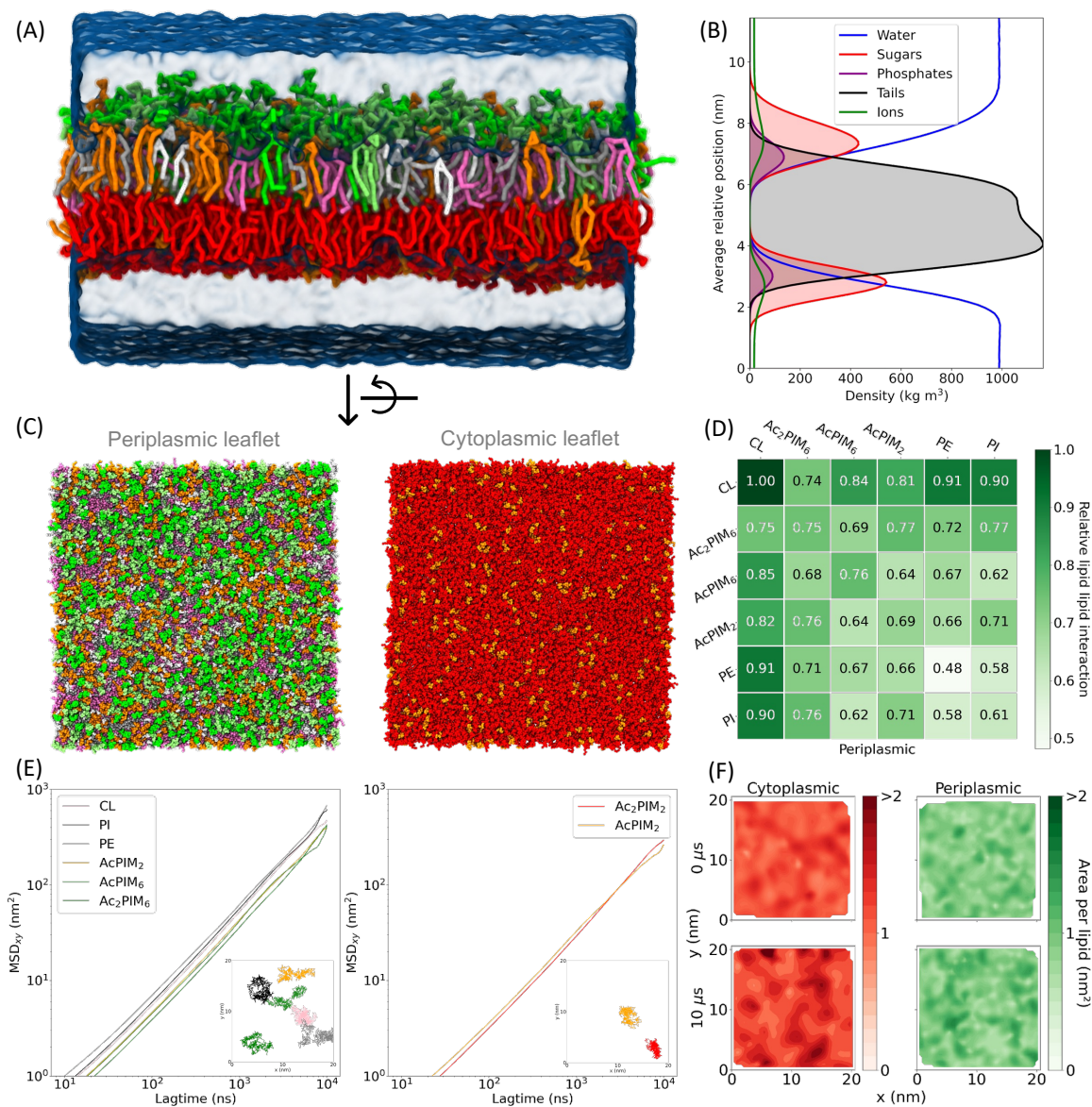
691 **Abbreviations**

692	AT:	Atomistic
693	ATP:	Adenosine triphosphate
694	BDQ:	Bedaquiline
695	CG:	Coarse grained
696	CL:	Cardiolipin
697	ISZ:	Isoniazid
698	LAM:	Lipoarabinomannan
699	LM:	Lipomannan
700	MD:	Molecular dynamics
701	<i>Mtb</i> :	<i>Mycobacterium tuberculosis</i>
702	NMR:	Nuclear magnetic resonance
703	PC:	Phosphatidylcholine
704	PE:	Phosphatidylethanolamine
705	PG:	Phosphatidylglycerol
706	PI:	Phosphatidylinositol
707	PIMs:	Phosphatidyl- <i>myo</i> -inositol mannosides
708	PIPs:	Phosphatidylinositol phosphates
709	PME:	Particle mesh Ewald
710	PMF:	Potential of mean force
711	TB:	Tuberculosis
712	TMM:	Trehalose monomycolate
713	VdW:	van der Waal
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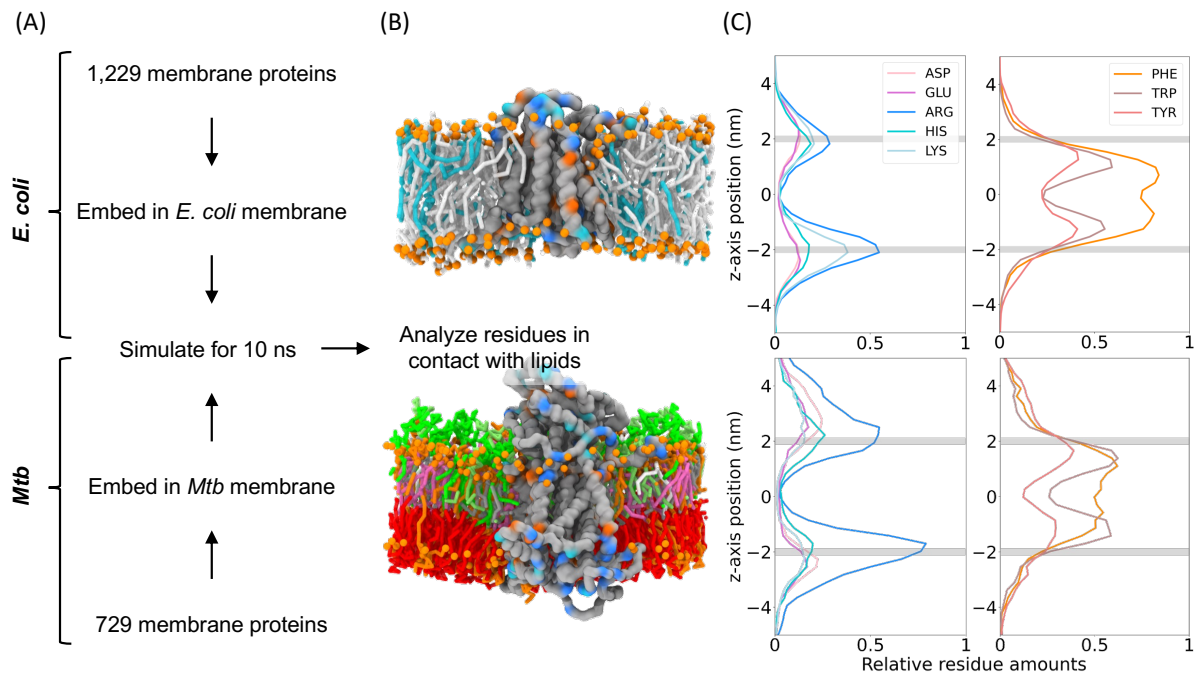
Figure 1: The structure of the mycobacterial lipids. **(A)** Schematic of an asymmetrical model of the mycobacterial plasma membrane and composition as previously defined (13). **(B)** Schematic of the core of the PIM lipids found in mycobacteria with the groupings for coarse-grained (CG) beads. The inositol core is highlighted in red. The bead types for MARTINI 3 are shown. **(C)** Overlay of the AT (sticks) and CG (spheres) models for each lipid, with chemical characteristics shown to the right.



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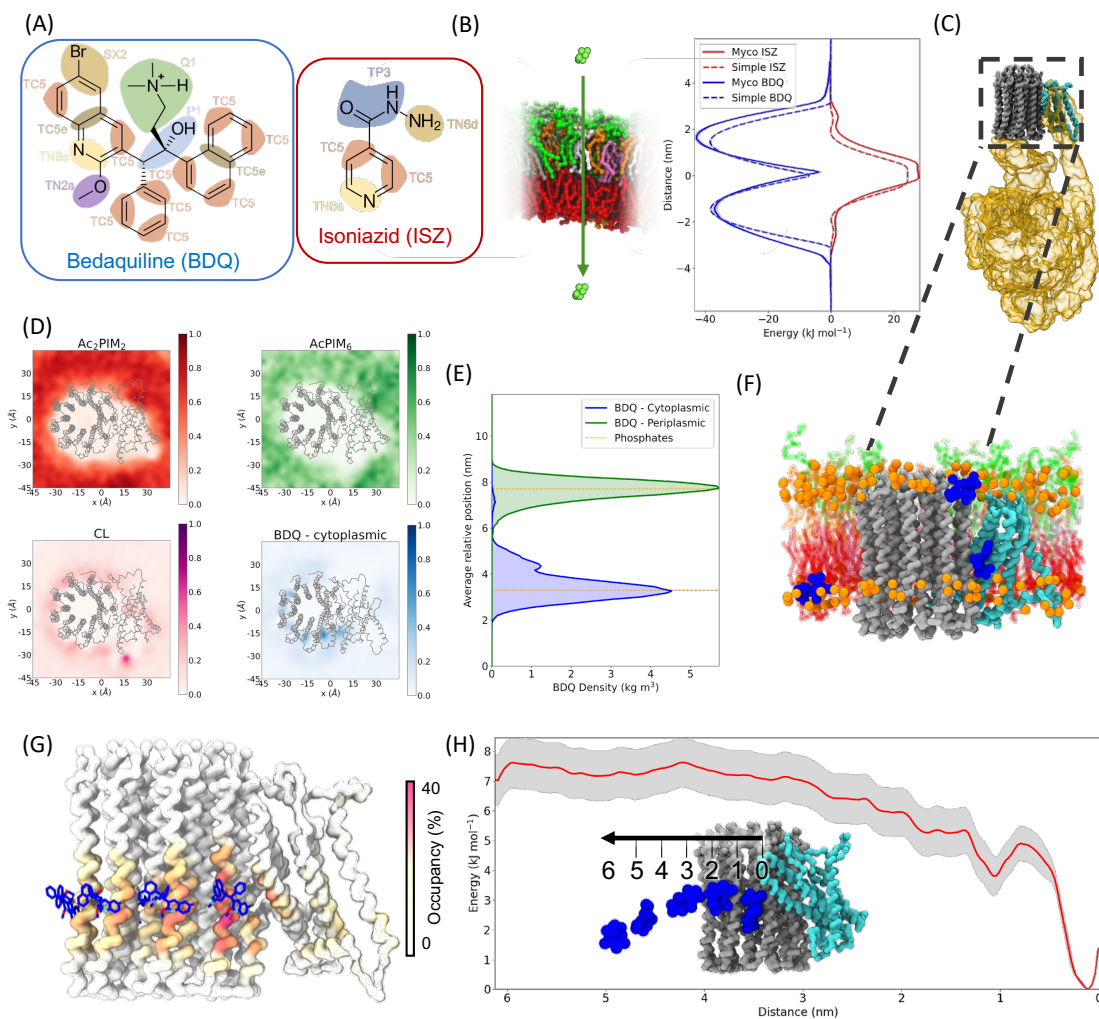
724 **Figure 2:** A mycobacterial membrane model. **(A)** Side view of the membrane with each lipid
 725 type depicted in a different color as shown in **Figure 1A**. **(B)** A density plot showing the density
 726 of water, sugar groups, phosphate groups, tail groups and ions over the simulation box. **(C)**
 727 Snapshots of the periplasmic and cytoplasmic leaflets whose compositions are: AcPIM₂ 22%,
 728 AcPIM₆ 11%, Ac₂PIM₆ 10%, CL 24%, PE 20% and PI 13% (periplasmic leaflet) and AcPIM₂
 729 10% and Ac₂PIM₂ 90% (cytoplasmic leaflet). The system size is 50 x 50 x 15 nm. **(D)** Relative
 730 number of neighbors of each lipid type for the periplasmic membrane. **(E)** Mean squared
 731 displacement (MSD_{xy}) (nm²) as a function of lagtime (ns) for each lipid type in the periplasmic
 732 and cytoplasmic leaflets. The inserts show the position of the phosphate group of each lipid
 733 type over the last 500 ns of the simulation. **(F)** Contour plots of the area per lipid (nm²) in each
 734 leaflet at the starting frame (upper) and final frame (lower) of the simulation. A darker color
 735 indicates a larger area per lipid.

736



737

738 **Figure 3:** The asymmetry of the mycobacterial membrane. **(A)** A simplified workflow for the
 739 lipid contacts analysis from all membrane proteins. **(B)** An example of a protein (*E. coli* protein
 740 *MraY* UniProt id: P0A6W3 and *Mtb* transporter *MmpL3* UniProt id: P9WJV5) embedded in
 741 either an *E. coli* or mycobacterial membrane. The orange spheres represent phosphates. For
 742 *E. coli* membrane the cyan sticks show PG and the grey sticks PE. For mycobacterial
 743 membrane, the lipid sticks are shown in the colors illustrated in **Figure 1A**. Selected residues
 744 are colored according to the key in **(C)**. **(C)** Graphs for *E. coli* and *Mtb* (top and bottom
 745 respectively) showing the relative abundance of selected residues within 8 Å of lipids. The
 746 gray lines show the position of the phosphates. The cytoplasmic region is shown by negative
 747 z values, the periplasmic region by positive values.
 748



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751 **Figure 4:** The behavior of the antibiotics with mycobacterial membrane and proteins. (A)
 752 Chemical structures of BDQ and ISZ with the CG groupings overlaid and bead types shown.
 753 (B) PMFs of the two antibiotics being pulled through either a mycobacterial or simple
 754 membrane in the z-direction. BDQ is shown in blue and ISZ in red, with the mycobacterial
 755 membrane results having a solid line and simple membrane having a dashed line. The error
 756 is shown in grey. A schematic of the PMF is shown to the left. (C) Structure of *Mycobacterium*
 757 *smegmatis* ATP synthase (PDB: 7JG5) with the c-subunits shown in grey, the a-subunit shown
 758 in cyan, and the other components shown as a gold surface. (D) Density in the x and y
 759 dimensions of selected lipids and BDQ when starting in the cytoplasmic leaflet relative to the
 760 protein shown in grey. (E) Density of the phosphates (orange) and BDQ over the course of
 761 the simulations where the antibiotic started in either the periplasmic leaflet (green) or the
 762 cytoplasmic leaflet (blue). (F) Snapshot of a single simulation containing a *Mtb* ATPase model
 763 and 8 x BDQ models showing the main positions BDQ occupied. Phosphates are shown in
 764 orange, BDQ shown in blue, c-subunits are shown in grey, and the a-subunit is shown in cyan.
 765 The lipid sticks are shown in the colors illustrated in **Figure 1A**. (G) Comparison of the highest
 766 occupancy sites identified with PyLipID (surface) and BDQ from the Cryo-EM structure (PDB:
 767 7JGC) (sticks). (H) PMF of BDQ moving through a mycobacterial plasma membrane with the
 768 error shown in grey. A schematic of the PMF is shown as an insert.

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771 **References**

772

- 773 1. WHO (2022) Global tuberculosis report 2022. (World Health Organization, Geneva).
774 2. C. R. Horsburgh, C. E. Barry, C. Lange, Treatment of Tuberculosis. *New England Journal*
775 *of Medicine* **373**, 2149-2160 (2015).
776 3. WHO (2021) Global Tuberculosis Report 2021. (World Health Organization, Geneva).
777 4. WHO (2022) The End TB Strategy.
778 5. WHO (2021) 1.4 million with tuberculosis, lost out on treatment during first year of
779 COVID-19.
780 6. L. Chiaradia *et al.*, Dissecting the mycobacterial cell envelope and defining the
781 composition of the native mycomembrane. *Scientific Reports* **7**, 12807 (2017).
782 7. C. L. Dulberger, E. J. Rubin, C. C. Boutte, The mycobacterial cell envelope — a moving
783 target. *Nature Reviews Microbiology* **18**, 47-59 (2020).
784 8. S. M. Batt, D. E. Minnikin, G. S. Besra, The thick waxy coat of mycobacteria, a protective
785 layer against antibiotics and the host's immune system. *Biochemical Journal* **477**,
786 1983-2006 (2020).
787 9. E. Fullam, R. J. Young, Physicochemical properties and Mycobacterium tuberculosis
788 transporters: keys to efficacious antitubercular drugs? *RSC Medicinal Chemistry* **12**,
789 43-56 (2021).
790 10. K. A. Sacksteder, M. Protopopova, C. E. Barry, 3rd, K. Andries, C. A. Nacy, Discovery
791 and development of SQ109: a new antitubercular drug with a novel mechanism of
792 action. *Future Microbiol* **7**, 823-837 (2012).
793 11. Y.-M. Boudehen *et al.*, Mycobacterial resistance to zinc poisoning requires assembly
794 of P-ATPase-containing membrane metal efflux platforms. *Nature Communications*
795 **13**, 4731 (2022).
796 12. J. M. Hayashi *et al.*, Spatially distinct and metabolically active membrane domain in
797 mycobacteria. *Proc Natl Acad Sci U S A* **113**, 5400-5405 (2016).
798 13. R. Bansal-Mutalik, H. Nikaido, Mycobacterial outer membrane is a lipid bilayer and the
799 inner membrane is unusually rich in diacyl phosphatidylinositol dimannosides.
800 *Proceedings of the National Academy of Sciences* **111**, 4958-4963 (2014).
801 14. M. Gilleron, V. F. J. Quesniaux, G. Puzo, Acylation State of the Phosphatidylinositol
802 Hexamannosides from *Mycobacterium bovis* Bacillus Calmette Guérin and
803 *Mycobacterium tuberculosis* H37Rv and Its Implication in Toll-like Receptor Response
804 *Journal of Biological Chemistry* **278**, 29880-29889 (2003).
805 15. M. Gilleron *et al.*, Acylation State of the Phosphatidylinositol Mannosides from
806 *Mycobacterium bovis* Bacillus Calmette Guérin and Ability to Induce Granuloma and
807 Recruit Natural Killer T Cells. *Journal of Biological Chemistry* **276**, 34896-34904 (2001).
808 16. M. Daffé, H. Marrakchi, Unraveling the Structure of the Mycobacterial Envelope.
809 *Microbiol Spectr* **7** (2019).
810 17. M. Jackson, The mycobacterial cell envelope-lipids. *Cold Spring Harb Perspect Med* **4**,
811 a021105 (2014).
812 18. R. Kalscheuer *et al.*, The Mycobacterium tuberculosis capsule: a cell structure with key
813 implications in pathogenesis. *Biochemical Journal* **476**, 1995-2016 (2019).
814 19. M. E. Guerin, J. Korduláková, P. M. Alzari, P. J. Brennan, M. Jackson, Molecular Basis
815 of Phosphatidyl-*myo*-inositol Mannoside Biosynthesis and Regulation in Mycobacteria
816 *Journal of Biological Chemistry* **285**, 33577-33583 (2010).

- 817 20. V. Corradi *et al.*, Emerging Diversity in Lipid–Protein Interactions. *Chemical Reviews*
818 **119**, 5775-5848 (2019).
- 819 21. R. A. Corey *et al.*, Identification and assessment of cardiolipin interactions with E. coli
820 inner membrane proteins. *Sci Adv* **7** (2021).
- 821 22. H. I. Ingólfsson *et al.*, Lipid Organization of the Plasma Membrane. *Journal of the*
822 *American Chemical Society* **136**, 14554-14559 (2014).
- 823 23. I. D. Pogozheva *et al.*, Comparative Molecular Dynamics Simulation Studies of Realistic
824 Eukaryotic, Prokaryotic, and Archaeal Membranes. *Journal of Chemical Information*
825 *and Modeling* **62**, 1036-1051 (2022).
- 826 24. P. Adhyapak *et al.*, Lipid Clustering in Mycobacterial Cell Envelope Layers Governs
827 Spatially Resolved Solvation Dynamics. *Chemistry – An Asian Journal* **17**, e202200146
828 (2022).
- 829 25. H. Guo *et al.*, Structure of mycobacterial ATP synthase bound to the tuberculosis drug
830 bedaquiline. *Nature* **589**, 143-147 (2021).
- 831 26. P. C. T. Souza *et al.*, Martini 3: a general purpose force field for coarse-grained
832 molecular dynamics. *Nature Methods* **18**, 382-388 (2021).
- 833 27. R. Alessandri *et al.*, Martini 3 Coarse-Grained Force Field: Small Molecules. *Advanced*
834 *Theory and Simulations* **5**, 2100391 (2022).
- 835 28. L. Borges-Araújo, P. C. T. Souza, F. Fernandes, M. N. Melo, Improved Parameterization
836 of Phosphatidylinositide Lipid Headgroups for the Martini 3 Coarse-Grain Force Field.
837 *Journal of Chemical Theory and Computation* **18**, 357-373 (2022).
- 838 29. P. Banerjee, R. Lipowsky, M. Santer, Coarse-Grained Molecular Model for the
839 Glycosylphosphatidylinositol Anchor with and without Protein. *Journal of Chemical*
840 *Theory and Computation* **16**, 3889-3903 (2020).
- 841 30. T. A. Wassenaar, H. I. Ingólfsson, R. A. Böckmann, D. P. Tieleman, S. J. Marrink,
842 Computational Lipidomics with insane: A Versatile Tool for Generating Custom
843 Membranes for Molecular Simulations. *Journal of Chemical Theory and Computation*
844 **11**, 2144-2155 (2015).
- 845 31. G. Bussi, D. Donadio, M. Parrinello, Canonical sampling through velocity rescaling. *J*
846 *Chem Phys* **126**, 014101 (2007).
- 847 32. M. Parrinello, A. Rahman, Polymorphic transitions in single crystals: A new molecular
848 dynamics method. **52:12** (1981).
- 849 33. M. J. Abraham *et al.*, GROMACS: High performance molecular simulations through
850 multi-level parallelism from laptops to supercomputers. *SoftwareX* **1-2**, 19-25 (2015).
- 851 34. J. A. Barker, R. O. Watts, Monte Carlo studies of the dielectric properties of water-like
852 models. *Molecular Physics* **26**, 789-792 (1973).
- 853 35. E. L. Wu, Y. Qi, K. C. Song, J. B. Klauda, W. Im, Preferred Orientations of
854 Phosphoinositides in Bilayers and Their Implications in Protein Recognition
855 Mechanisms. *The Journal of Physical Chemistry B* **118**, 4315-4325 (2014).
- 856 36. J. Lee *et al.*, CHARMM-GUI Membrane Builder for Complex Biological Membrane
857 Simulations with Glycolipids and Lipoglycans. *Journal of Chemical Theory and*
858 *Computation* **15**, 775-786 (2019).
- 859 37. K. Vanommeslaeghe *et al.*, CHARMM general force field: A force field for drug-like
860 molecules compatible with the CHARMM all-atom additive biological force fields.
861 *Journal of computational chemistry* **31**, 671-690 (2010).
- 862 38. J. Huang *et al.*, CHARMM36m: an improved force field for folded and intrinsically
863 disordered proteins. *Nature Methods* **14**, 71-73 (2017).

- 864 39. E. L. Wu *et al.*, CHARMM-GUI Membrane Builder toward realistic biological membrane
865 simulations. *Journal of Computational Chemistry* **35**, 1997-2004 (2014).
- 866 40. U. Essmann *et al.*, A smooth particle mesh Ewald method. *The Journal of Chemical*
867 *Physics* **103**, 8577-8593 (1995).
- 868 41. S.-J. Marrink (2022) Downloads: Lipids.
- 869 42. B. M. Groenewald W, Croft A, Marrink S-J., Molecular Dynamics of Mycolic Acid
870 Monolayers. *ChemRxiv* (2019).
- 871 43. P. W. Fowler *et al.*, Membrane stiffness is modified by integral membrane proteins.
872 *Soft Matter* **12**, 7792-7803 (2016).
- 873 44. P. Smith, C. D. Lorenz, LiPyphilic: A Python Toolkit for the Analysis of Lipid Membrane
874 Simulations. *Journal of Chemical Theory and Computation* **17**, 5907-5919 (2021).
- 875 45. M. Bonomi *et al.*, Promoting transparency and reproducibility in enhanced molecular
876 simulations. *Nature Methods* **16**, 670-673 (2019).
- 877 46. S. Buchoux, FATSLiM: a fast and robust software to analyze MD simulations of
878 membranes. *Bioinformatics* **33**, 133-134 (2017).
- 879 47. J. D. Hunter, Matplotlib: A 2D Graphics Environment. *Computing in Science &*
880 *Engineering* **9**, 90-95 (2007).
- 881 48. C. UniProt, UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res*
882 **49**, D480-D489 (2021).
- 883 49. J. Jumper *et al.*, Highly accurate protein structure prediction with AlphaFold. *Nature*
884 **596**, 583-589 (2021).
- 885 50. T. Nugent, D. T. Jones, Membrane protein orientation and refinement using a
886 knowledge-based statistical potential. *BMC Bioinformatics* **14**, 276 (2013).
- 887 51. P. C. Kroon (2020) Aggregate, automate, assemble. . (University of Groningen), pp 16-
888 53.
- 889 52. J. A. Graham, J. W. Essex, S. Khalid, PyCGTOOL: Automated Generation of Coarse-
890 Grained Molecular Dynamics Models from Atomistic Trajectories. *Journal of Chemical*
891 *Information and Modeling* **57**, 650-656 (2017).
- 892 53. S. Kim *et al.*, CHARMM-GUI ligand reader and modeler for CHARMM force field
893 generation of small molecules. *Journal of Computational Chemistry* **38**, 1879-1886
894 (2017).
- 895 54. N. C. f. B. Information (2022) PubChem Compound Summary for CID 5388906,
896 Bedaquiline. (National Center for Biotechnology Information).
- 897 55. I. V. Tetko *et al.*, Virtual Computational Chemistry Laboratory – Design and
898 Description. *Journal of Computer-Aided Molecular Design* **19**, 453-463 (2005).
- 899 56. A. Waterhouse *et al.*, SWISS-MODEL: homology modelling of protein structures and
900 complexes. *Nucleic Acids Research* **46**, W296-W303 (2018).
- 901 57. F. Sievers *et al.*, Fast, scalable generation of high-quality protein multiple sequence
902 alignments using Clustal Omega. *Molecular Systems Biology* **7**, 539 (2011).
- 903 58. W. Song *et al.*, PyLipID: A Python Package for Analysis of Protein–Lipid Interactions
904 from Molecular Dynamics Simulations. *Journal of Chemical Theory and Computation*
905 **18**, 1188-1201 (2022).
- 906 59. R. A. Corey, O. N. Vickery, M. S. P. Sansom, P. J. Stansfeld, Insights into Membrane
907 Protein–Lipid Interactions from Free Energy Calculations. *Journal of Chemical Theory*
908 *and Computation* **15**, 5727-5736 (2019).

- 909 60. M. Souaille, B. t. Roux, Extension to the weighted histogram analysis method:
910 combining umbrella sampling with free energy calculations. *Computer Physics*
911 *Communications* **135**, 40-57 (2001).
- 912 61. J. S. Hub, B. L. de Groot, D. van der Spoel, g_wham—A Free Weighted Histogram
913 Analysis Implementation Including Robust Error and Autocorrelation Estimates.
914 *Journal of Chemical Theory and Computation* **6**, 3713-3720 (2010).
- 915 62. P. S. Schmalhorst, F. Deluweit, R. Scherrers, C.-P. Heisenberg, M. Sikora, Overcoming
916 the Limitations of the MARTINI Force Field in Simulations of Polysaccharides. *Journal*
917 *of Chemical Theory and Computation* **13**, 5039-5053 (2017).
- 918 63. C. Hoffmann, A. Leis, M. Niederweis, J. M. Plitzko, H. Engelhardt, Disclosure of the
919 mycobacterial outer membrane: Cryo-electron tomography and vitreous sections
920 reveal the lipid bilayer structure. *Proceedings of the National Academy of Sciences*
921 **105**, 3963-3967 (2008).
- 922 64. B. Zuber *et al.*, Direct visualization of the outer membrane of mycobacteria and
923 corynebacteria in their native state. *J Bacteriol* **190**, 5672-5680 (2008).
- 924 65. P. Adhyapak *et al.*, Dynamical Organization of Compositionally Distinct Inner and
925 Outer Membrane Lipids of Mycobacteria. *Biophysical Journal* **118**, 1279-1291 (2020).
- 926 66. A. V. Hughes *et al.*, Physical Properties of Bacterial Outer Membrane Models: Neutron
927 Reflectometry & Molecular Simulation. *Biophysical Journal* **116**, 1095-1104 (2019).
- 928 67. R. Friedman, Membrane-Ion Interactions. *The Journal of membrane biology* **251**, 453-
929 460 (2018).
- 930 68. B. Modak, S. Girkar, R. Narayan, S. Kapoor, Mycobacterial Membranes as Actionable
931 Targets for Lipid-Centric Therapy in Tuberculosis. *Journal of Medicinal Chemistry* **65**,
932 3046-3065 (2022).
- 933 69. P. P. Nguyen, T. Kado, M. Prithviraj, M. S. Siegrist, Y. S. Morita, Inositol acylation of
934 phosphatidylinositol mannosides: a rapid mass response to membrane fluidization in
935 mycobacteria. *Journal of Lipid Research* **63** (2022).
- 936 70. J. H. Lorent *et al.*, Plasma membranes are asymmetric in lipid unsaturation, packing
937 and protein shape. *Nat Chem Biol* **16**, 644-652 (2020).
- 938 71. G. von Heijne, Net N-C charge imbalance may be important for signal sequence
939 function in bacteria. *Journal of Molecular Biology* **192**, 287-290 (1986).
- 940 72. J. A. Baker, W.-C. Wong, B. Eisenhaber, J. Warwicker, F. Eisenhaber, Charged residues
941 next to transmembrane regions revisited: “Positive-inside rule” is complemented by
942 the “negative inside depletion/outside enrichment rule”. *BMC Biology* **15**, 66 (2017).
- 943 73. I. D. Pogozeva, S. Tristram-Nagle, H. I. Mosberg, A. L. Lomize, Structural adaptations
944 of proteins to different biological membranes. *Biochimica et Biophysica Acta (BBA) -*
945 *Biomembranes* **1828**, 2592-2608 (2013).
- 946 74. R. J. Anderson, P. W. Groundwater, A. Todd, A. J. Worsley, *Antibacterial agents :
947 chemistry, mode of action, mechanisms of resistance and clinical applications* (Wiley-
948 Blackwell, Chichester, 2012), pp. 378.
- 949 75. V. A. Dartois, E. J. Rubin, Anti-tuberculosis treatment strategies and drug
950 development: challenges and priorities. *Nature Reviews Microbiology*
951 10.1038/s41579-022-00731-y (2022).
- 952 76. A. Krah, G. Grüber, P. J. Bond, Binding properties of the anti-TB drugs bedaquiline and
953 TBAJ-876 to a mycobacterial F-ATP synthase. *Current Research in Structural Biology* **4**,
954 278-284 (2022).

- 955 77. F. Bardou, C. Raynaud, C. Ramos, M. A. Lan elle, G. Lan elle, Mechanism of isoniazid
956 uptake in Mycobacterium tuberculosis. *Microbiology* **144**, 2539-2544 (1998).
- 957 78. A. N. Unissa, S. Subbian, L. E. Hanna, N. Selvakumar, Overview on mechanisms of
958 isoniazid action and resistance in Mycobacterium tuberculosis. *Infection, Genetics and*
959 *Evolution* **45**, 474-492 (2016).
- 960 79. M. Jackson *et al.*, Inactivation of the antigen 85C gene profoundly affects the mycolate
961 content and alters the permeability of the Mycobacterium tuberculosis cell envelope.
962 *Molecular Microbiology* **31**, 1573-1587 (1999).
- 963 80. A. L. Duncan, A. J. Robinson, J. E. Walker, Cardiolipin binds selectively but transiently
964 to conserved lysine residues in the rotor of metazoan ATP synthases. *Proceedings of*
965 *the National Academy of Sciences* **113**, 8687-8692 (2016).
- 966 81. R. A. Corey, P. J. Stansfeld, M. S. P. Sansom, The energetics of protein-lipid interactions
967 as viewed by molecular simulations. *Biochem Soc Trans* **48**, 25-37 (2020).
- 968 82. J. Briffotiaux, W. Huang, X. Wang, B. Gicquel, MmpS5/MmpL5 as an efflux pump in
969 Mycobacterium species. *Tuberculosis* **107**, 13-19 (2017).
- 970 83. G. Degiacomi *et al.*, In vitro Study of Bedaquiline Resistance in Mycobacterium
971 tuberculosis Multi-Drug Resistant Clinical Isolates. *Frontiers in Microbiology* **11** (2020).
- 972 84. R. J. Clarke, K. R. Hossain, K. Cao, Physiological roles of transverse lipid asymmetry of
973 animal membranes. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1862**,
974 183382 (2020).
- 975 85. W. Im, S. Khalid, Molecular Simulations of Gram-Negative Bacterial Membranes Come
976 of Age. *Annual Review of Physical Chemistry* **71**, 171-188 (2020).
- 977 86. J. Augenreich *et al.*, The conical shape of DIM lipids promotes *Mycobacterium*
978 *tuberculosis* infection of macrophages. *Proceedings of the National Academy of*
979 *Sciences* **116**, 25649-25658 (2019).
- 980 87. A. Queiroz, L. W. Riley, Bacterial immunostat: Mycobacterium tuberculosis lipids and
981 their role in the host immune response. *Rev Soc Bras Med Trop* **50**, 9-18 (2017).
- 982 88. K. A. Abrahams, G. S. Besra, Synthesis and recycling of the mycobacterial cell envelope.
983 *Current Opinion in Microbiology* **60**, 58-65 (2021).
- 984