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Conformational Dynamics and Stability of Mycolic Acids Bilayers from the *Mycobacterium tuberculosis* Outer Membrane

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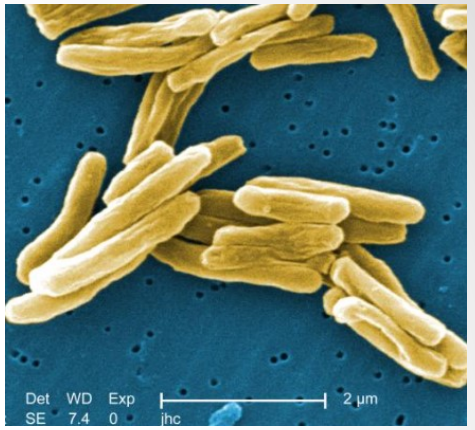
Drug-resistant tuberculosis (TB) is a new challenge to modern medicine

- TB is one of the most widespread and socially significant infections
- Every year, 1.6 million people die worldwide, making TB the leading cause of death from a single infectious agent
- TB is caused by the pathogenic *Mycobacterium tuberculosis* (MTB)
- New emerging strains of mycobacteria: drug-resistant and multidrug-resistant TB



During evolution, MTB developed special way for survival inside infected organism

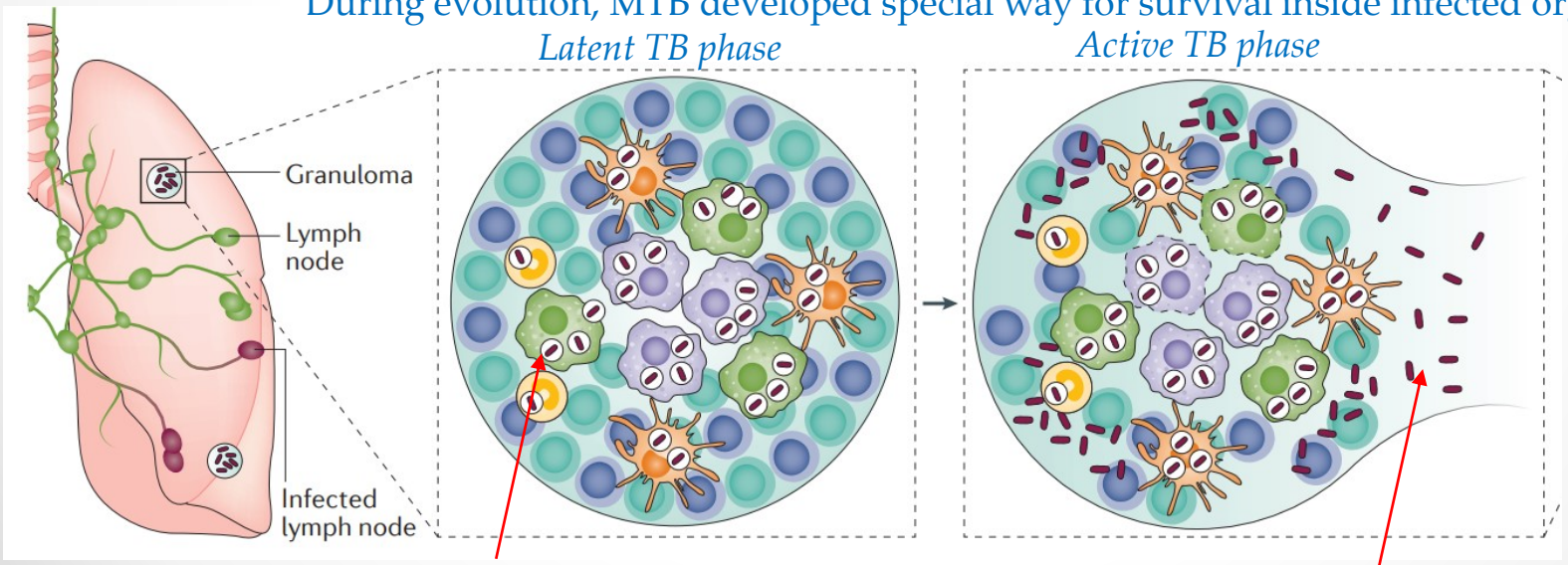


Figure from [Pai M. et al., *Nat Rev Dis Primers*, 2016, **2**,16076]

MTB survives inside macrophage "hiding" from drugs

If macrophage dies due to bad external conditions, MTB goes outside

Key factor of *MTB* resilience is its extremely complicated and persistent cell wall

Outer membrane of mycolic acids: long molecules with hydrocarbon chains of ~70-90 carbon atoms

"Normal" lipid membrane

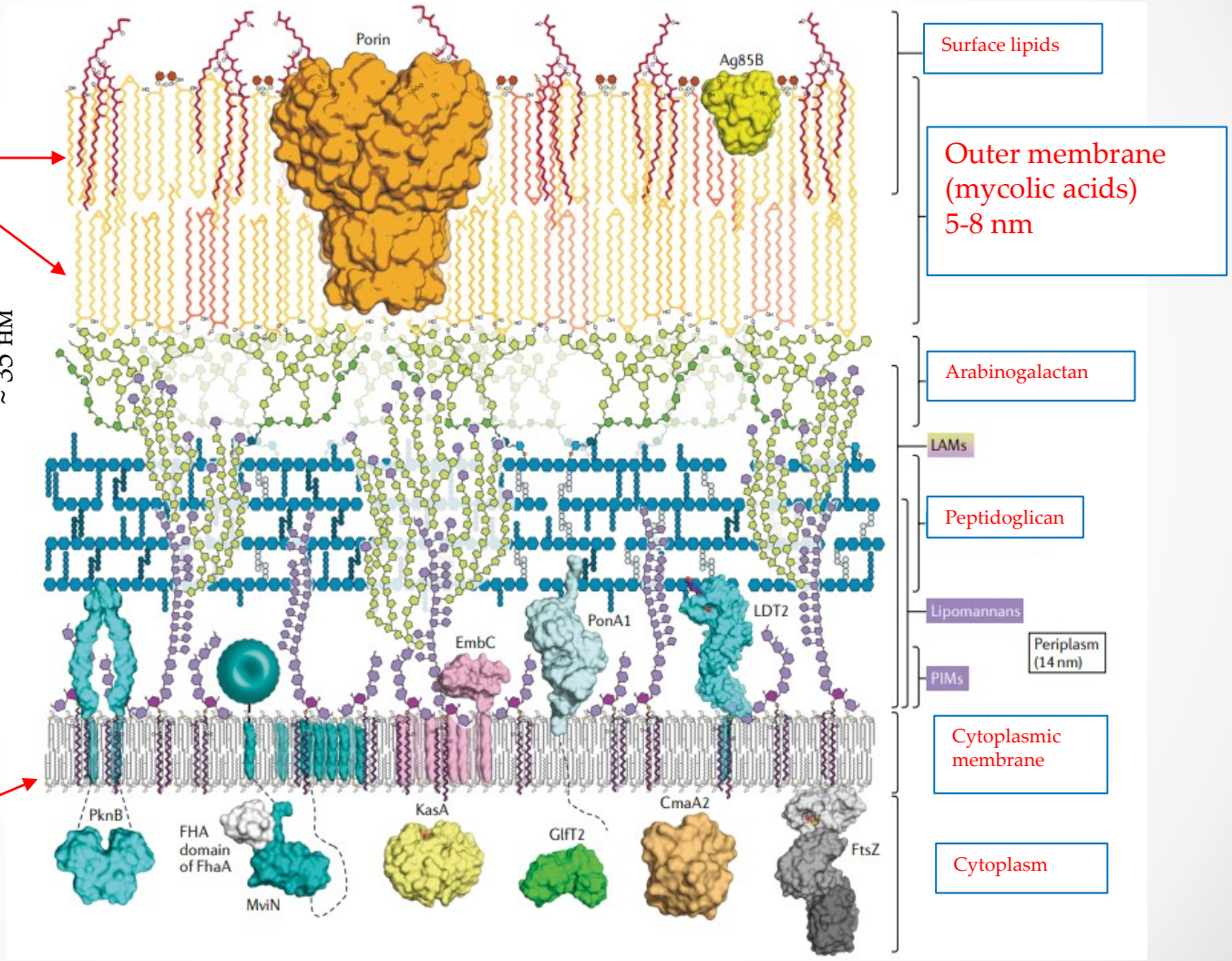
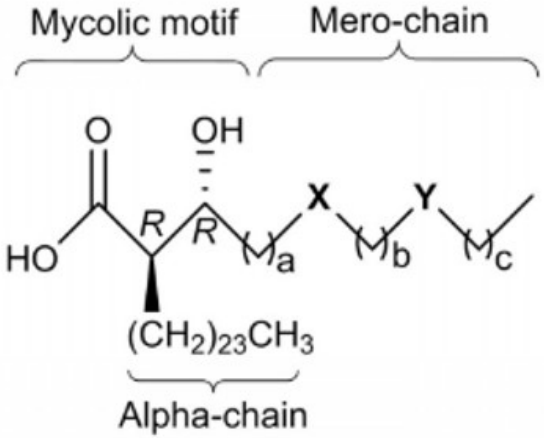


Figure from [Dulberger C.L.et al. *Nat Rev Microbiol,* 2020, **18**, 47]

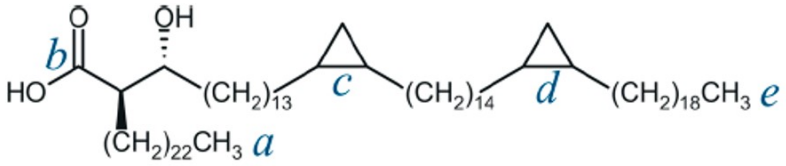
Mycolic acids – main components of *MTB* outer membrane



- Long molecules: 70-90 carbon atom chains
- Different length and X,Y groups in different organisms

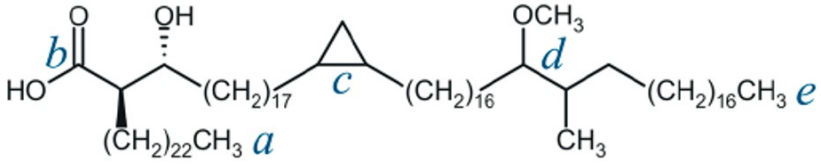
Main chemical compositions in MTB:

Alpha-MA



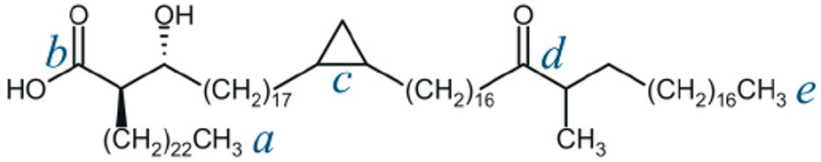
AMA

Methoxy-MA



MMA

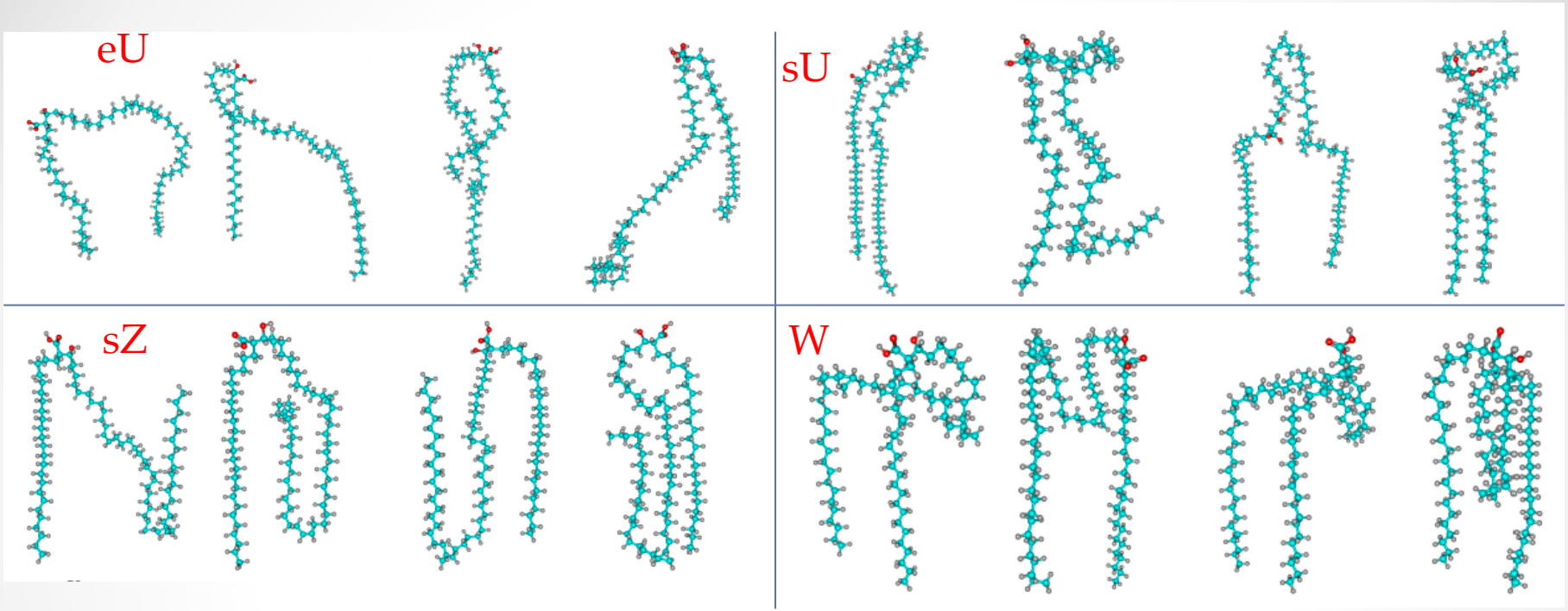
Keto-MA



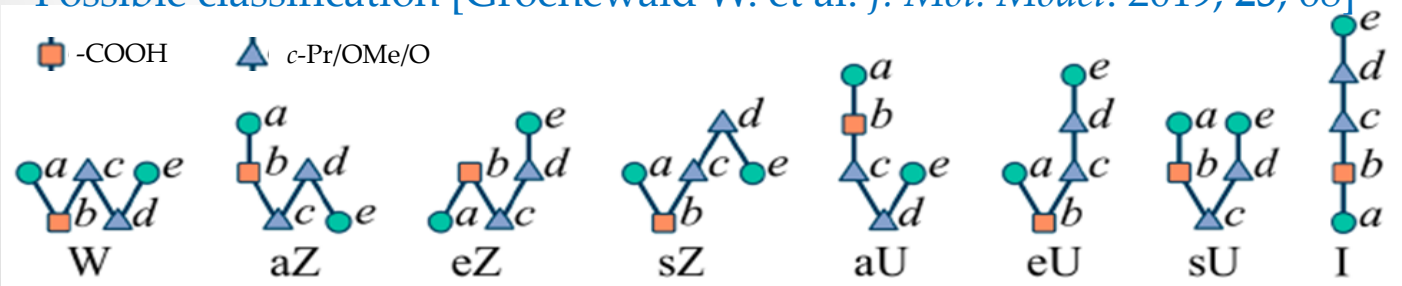
KMA

Very flexible molecules, different packings and conformations are feasible

Very flexible molecules, many conformations are feasible

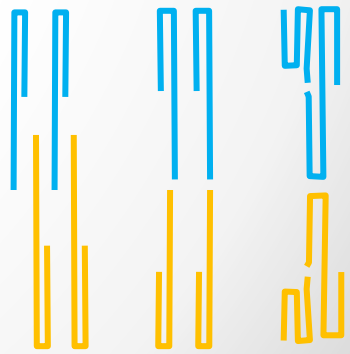


Possible classification [Groenewald W. et al. *J. Mol. Model*. 2019, **25**, 68]



Special algorithms are required for conformation recognition for millions of molecules

Packings:



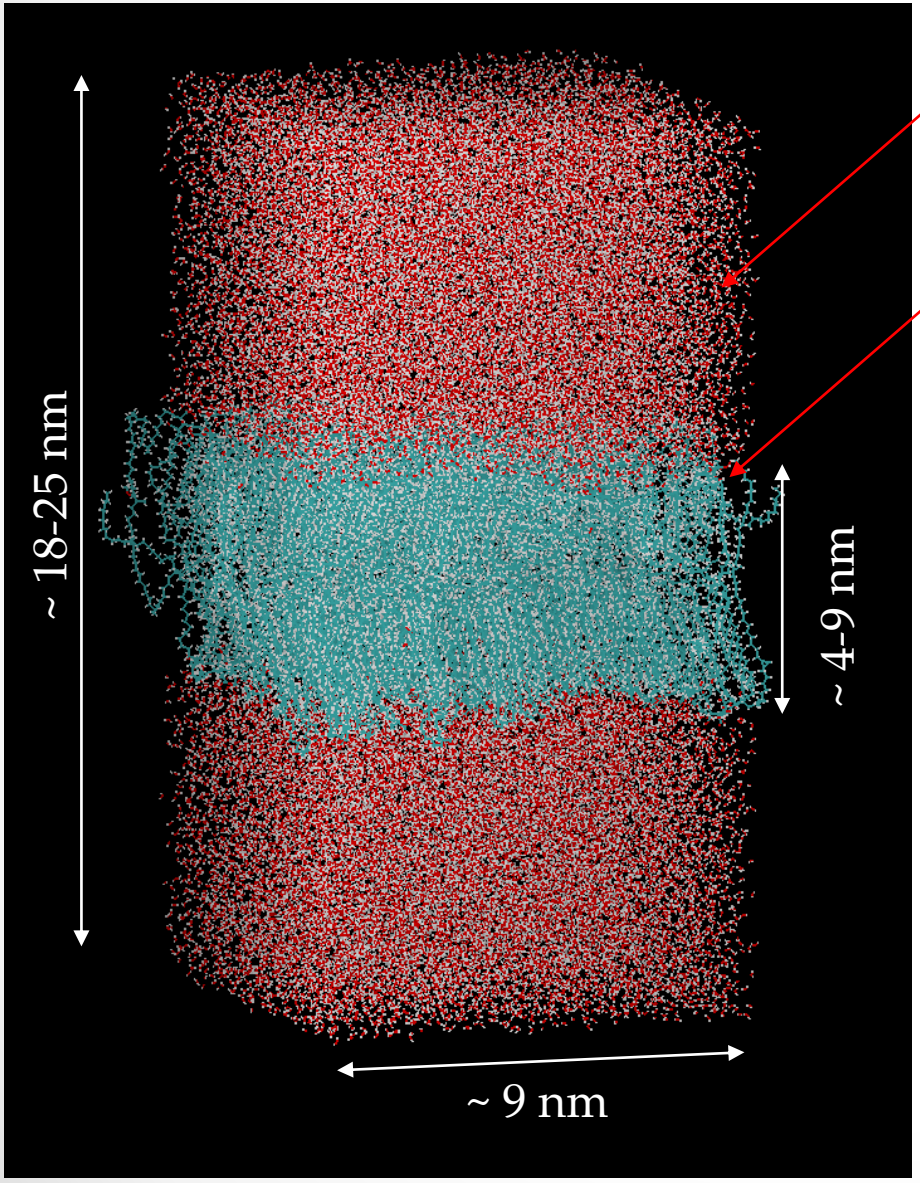
Problems and study goals:

- What is the preferred packing and conformational composition of MA in MTB membranes?
- How quickly can the restructuring of conformational composition and packaging occur?
- How do packaging method and conformational composition affect membrane properties (density, thickness, permittivity for drugs)?

More general questions:

- Could the regulation of membrane composition and packaging modes be a mechanism of MTB's struggle for survival?
- Is it possible to develop anti-MTB drugs that affect membrane properties and their regulation?

Method: MD simulations



Up to 150000 atoms in a system, solvation SPC water, T=300K

Membrane: 200 MA molecules
2 leaflets of 10x10 molecules

Various chemical and conformational compositions

All-atomic molecular dynamics: Charm36 force field, NPT ensemble, GROMACS 2022/2023, GPU support

MD trajectory: typically 300 ns, up to 1200 ns in some runs

Specially developed programs for conformations recognition

- Studied compositions:

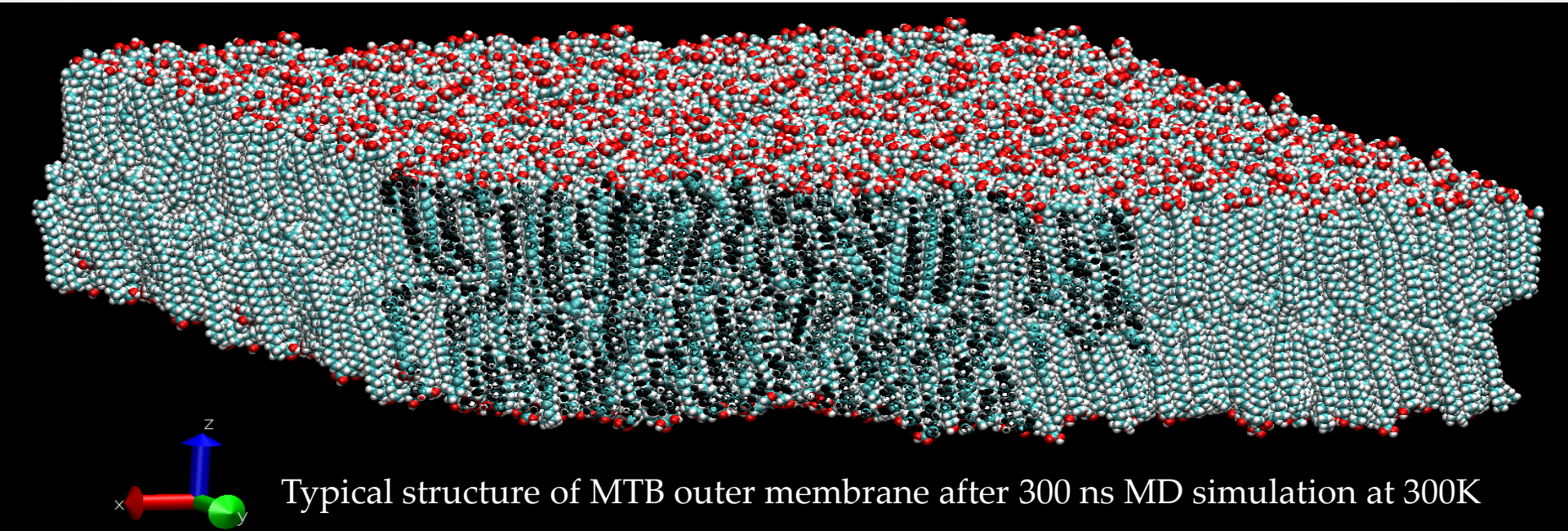
- Pure AMA, KMA, MMA
- Mixed30 – 70% of AMA (140AMA:30KMA:30MMA)
- Mixed50 – 50% of AMA (100 AMA:50KMA:50MMA)

- Initial conformational composition:

- Pure MA membranes: 100% W, sZ, sU, eU, or mixture of them
- Mixed membranes: W-W-W, eU-W-W

- Initial packings:

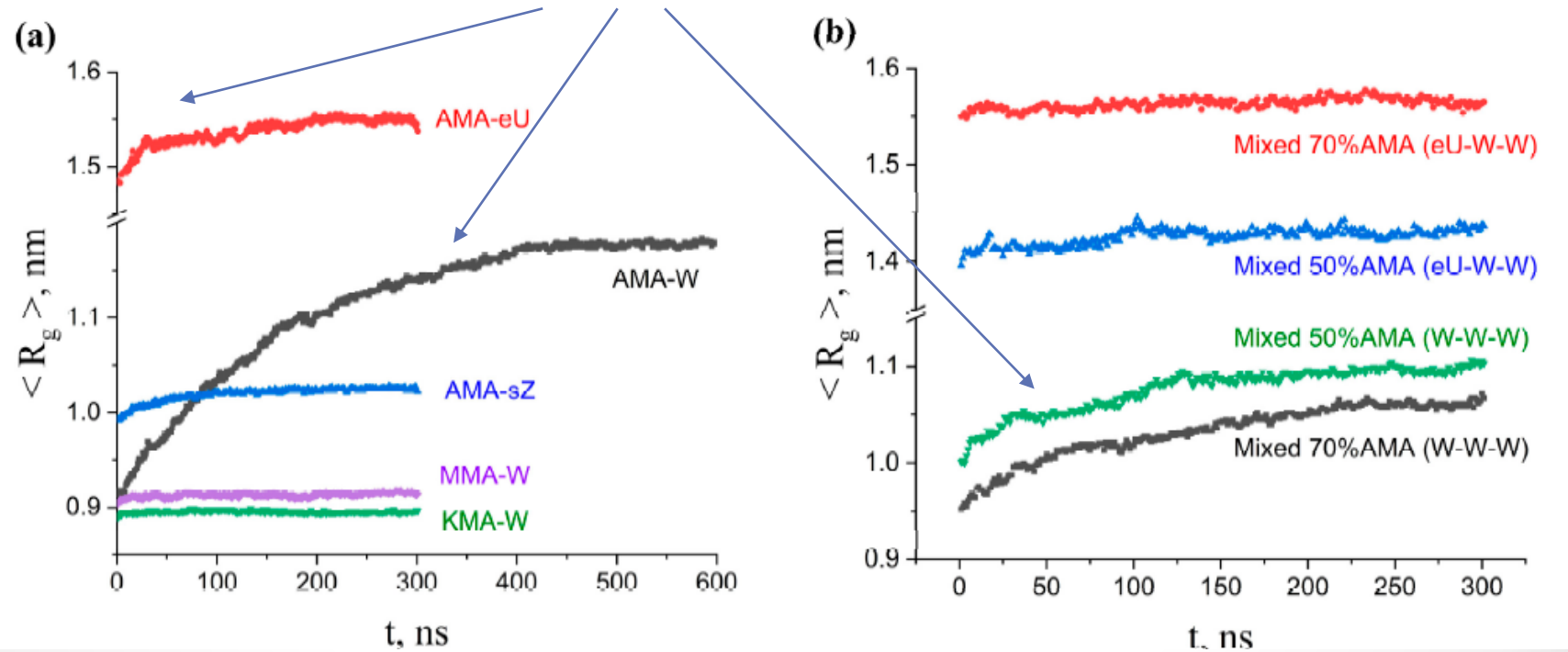
- Tail-to-tail
- Tails overlapped and interlaced



Typical structure of MTB outer membrane after 300 ns MD simulation at 300K

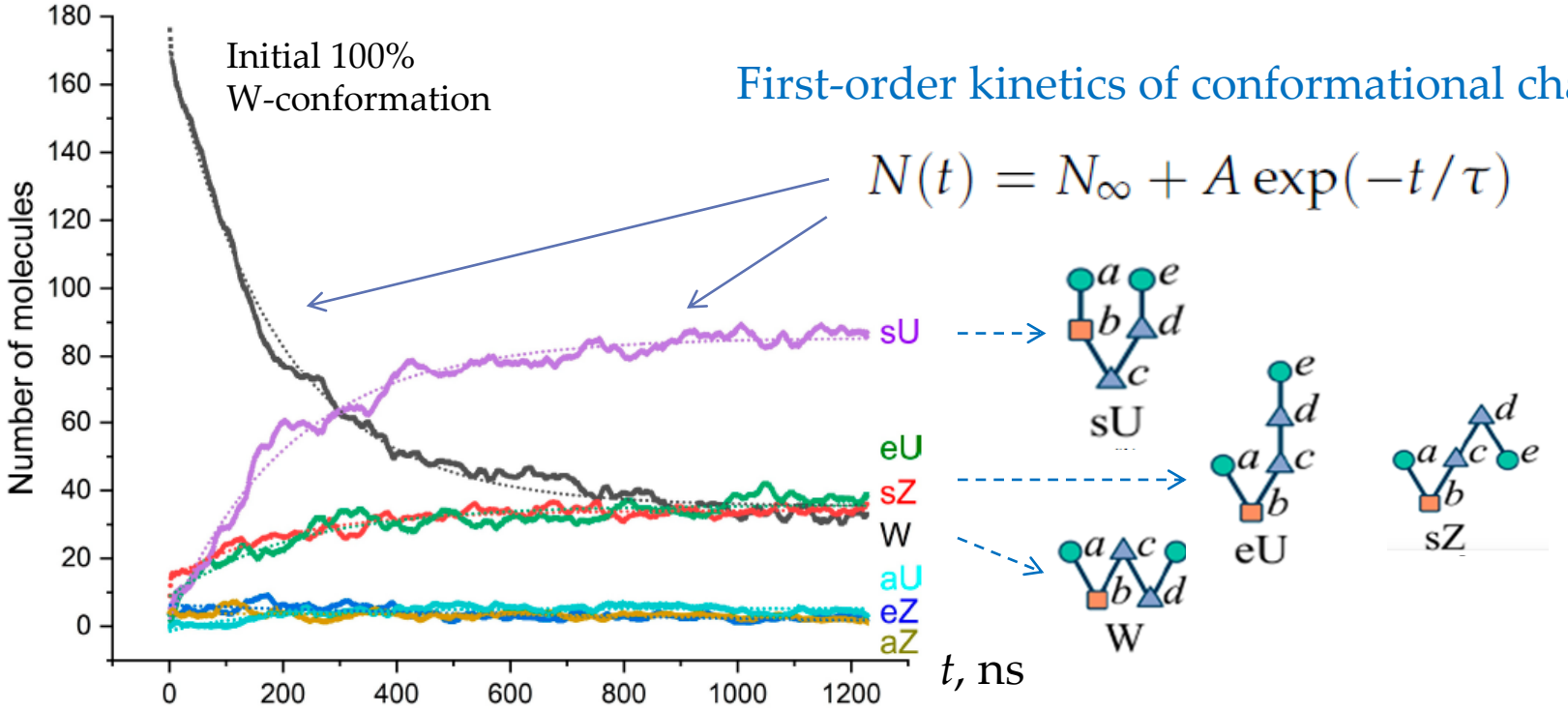
The conformational changes can be monitored using the average gyration radii of MA molecules

MA membranes undergo fast conformational changes in large extent (typical times of 100-300 ns)



Despite fast conformational changes, the packings "tail-to-tail" or "overlapped" are quite stable, there are no significant rearrangements between them for hundreds of nanoseconds

AMA-W membrane is most labile, it is quickly transformed to a large extent



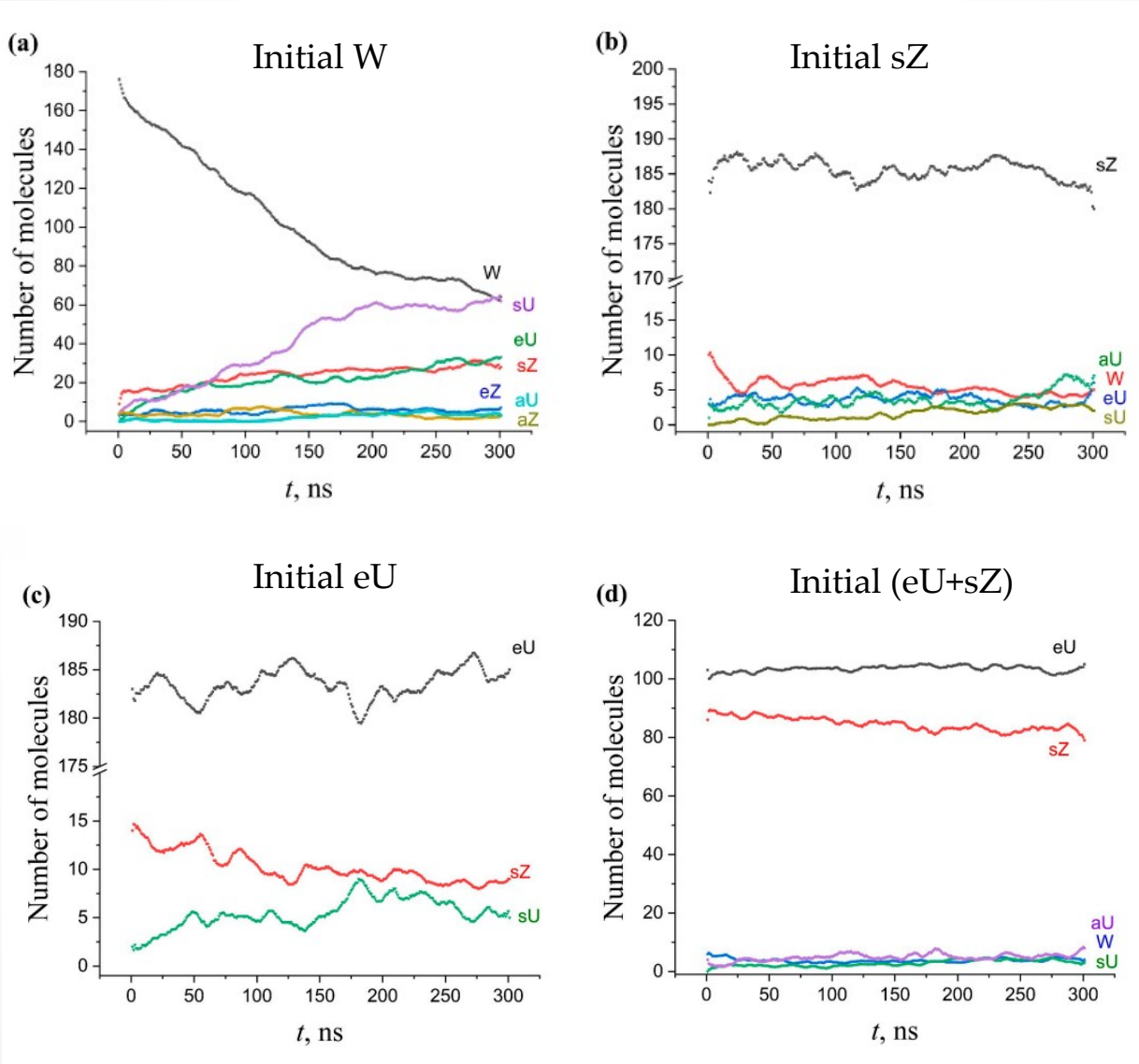
First-order kinetics of conformational changes:

$$N(t) = N_\infty + A\exp(-t/\tau)$$

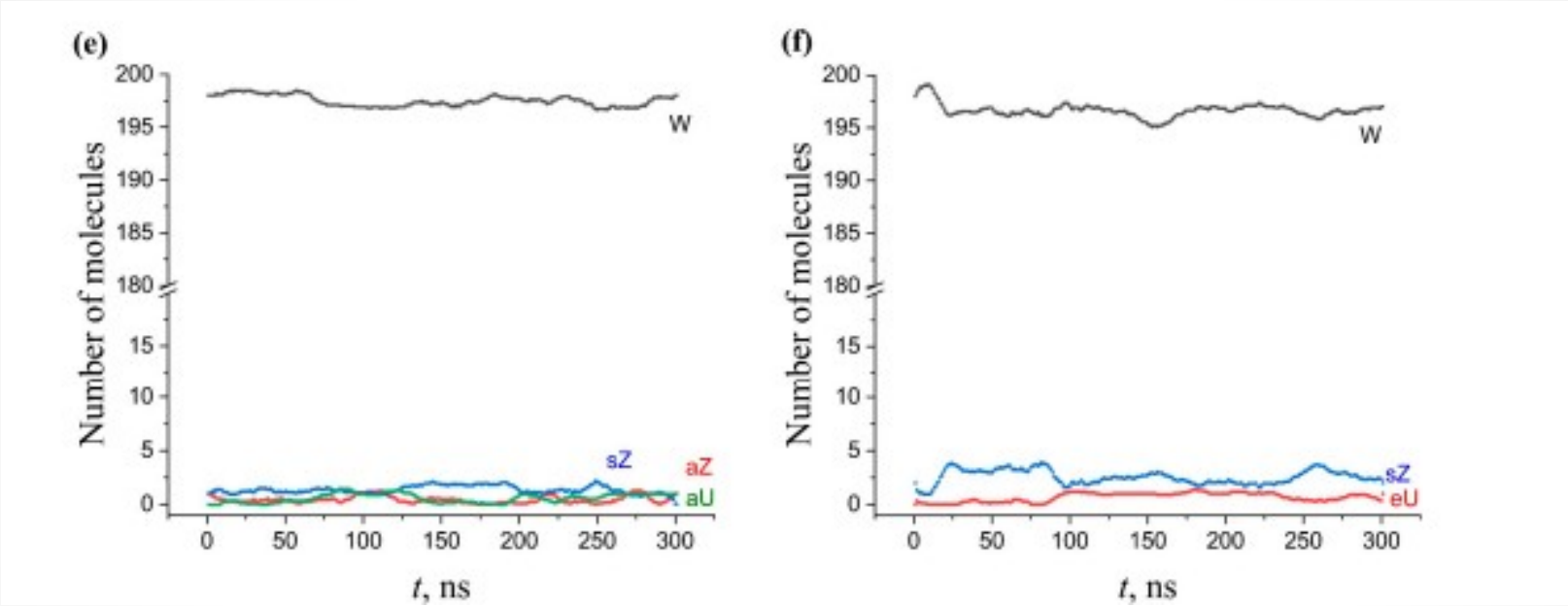
Kinetic parameters of conformational changes:

	W	sZ	eU	sU	aU
Final number of molecules N_{1200}	33	36	38	87	3
Equilibrium number of molecules N_∞	35.3 ± 0.1	34.2 ± 0.1	35.6 ± 0.1	85.6 ± 0.1	5.25 ± 0.04
Equilibrium fraction of molecules $x = N_\infty / N_{total}$	0.177	0.171	0.178	0.428	0.027
Characteristic time of accumulation/decay τ, ns	192 ± 0.1	198.5 ± 3.6	222.6 ± 5.8	220.1 ± 2.1	160.7 ± 6.6
Pre-exponential coefficient A	135.0 ± 0.4	-20.2 ± 0.2	-26.8 ± 0.3	-83.6 ± 0.4	-6.6 ± 0.2
Rate constant of accumulation/decay $(1/\tau) \cdot 10^{-6}$, s^{-1}	5.20	5.04	4.49	4.54	6.22
Coefficient of determination R^2	0.991	0.924	0.863	0.979	0.684

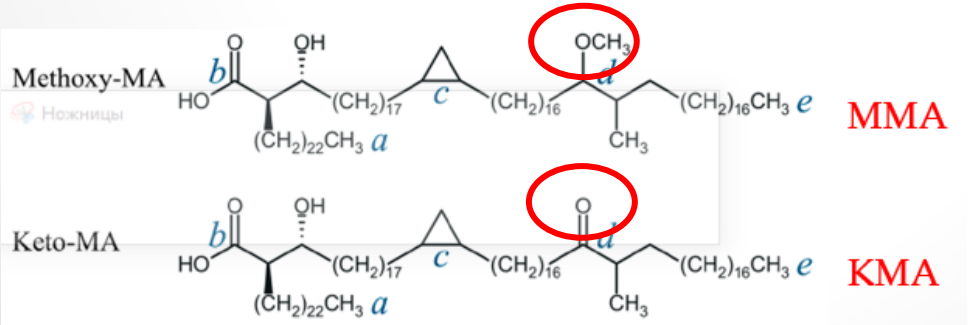
Other AMA membranes are more “stable”

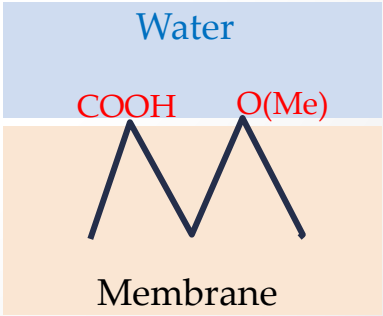


Especially stable (and favorable) W-conformations in pure KMA and MMA membranes

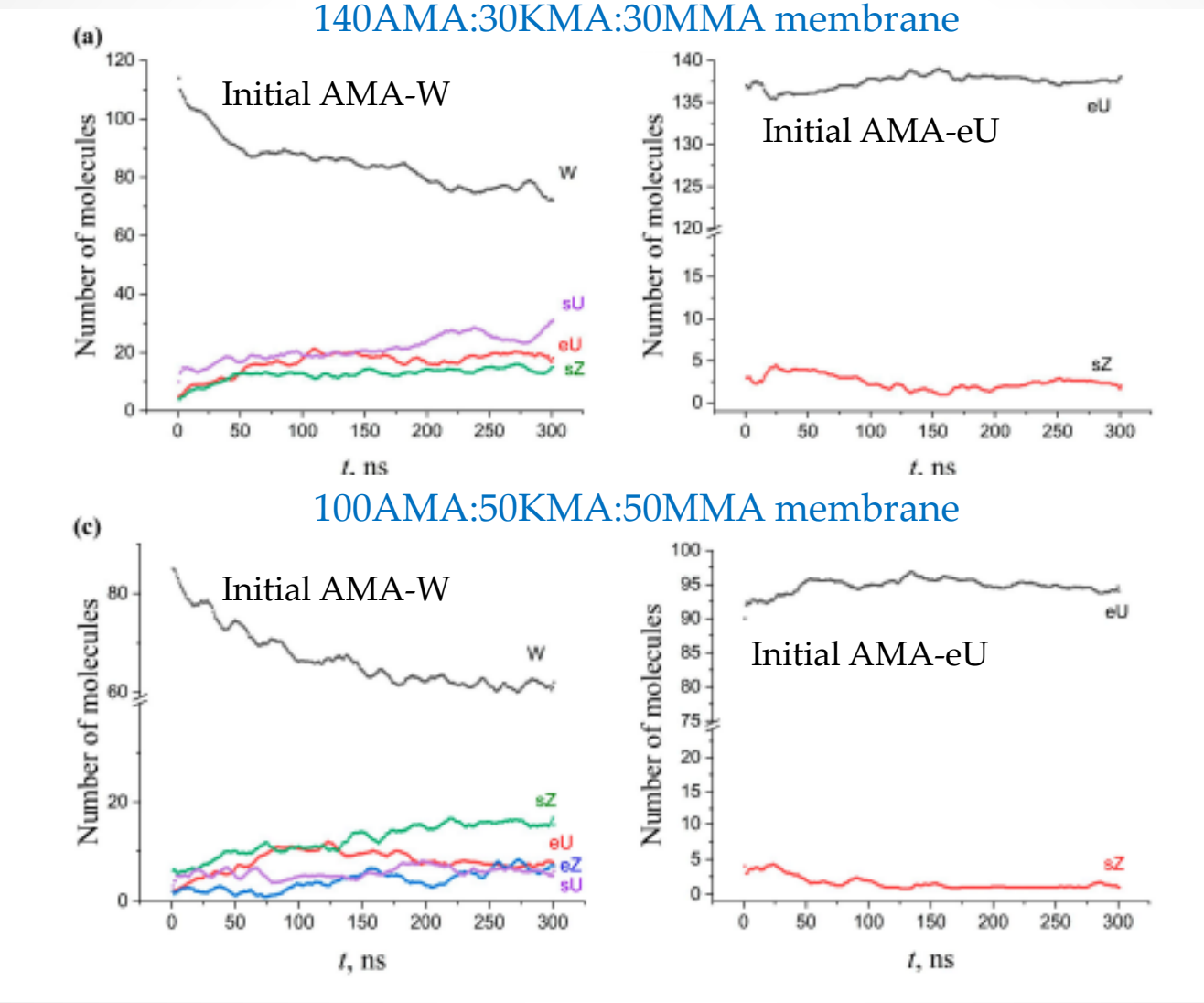


The origin of such stability – additional hydrofillic O-atoms favoring W-shapes in water:





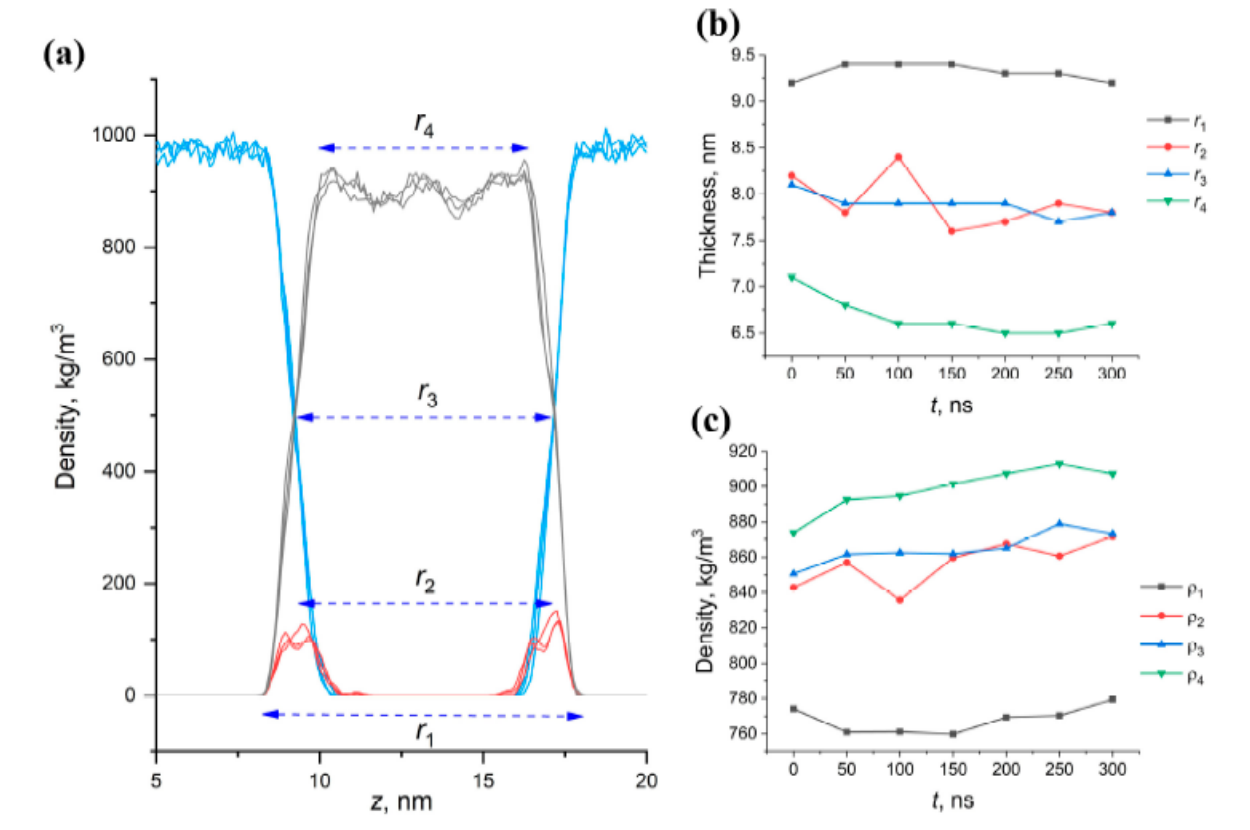
KMA and MMA molecules also stabilize AMA conformations in mixed membranes:



- Thus, KMA and MMA in the mycobacterial envelope play the role of a conformational stabilizer of AMA, increasing the stability of membranes.

For the protective properties of the membrane, its density and thickness are primarily important

Membrane thickness – different methods of estimation



r_3 was found to be the most reliable parameter

Thickness and density of membrane is crucially connected to the their chemical and conformation composition

Table 2. Thickness and density of the AMA-based and mixed membranes after 300 ns of NPT MD (parameters determined on the basis of r_3).

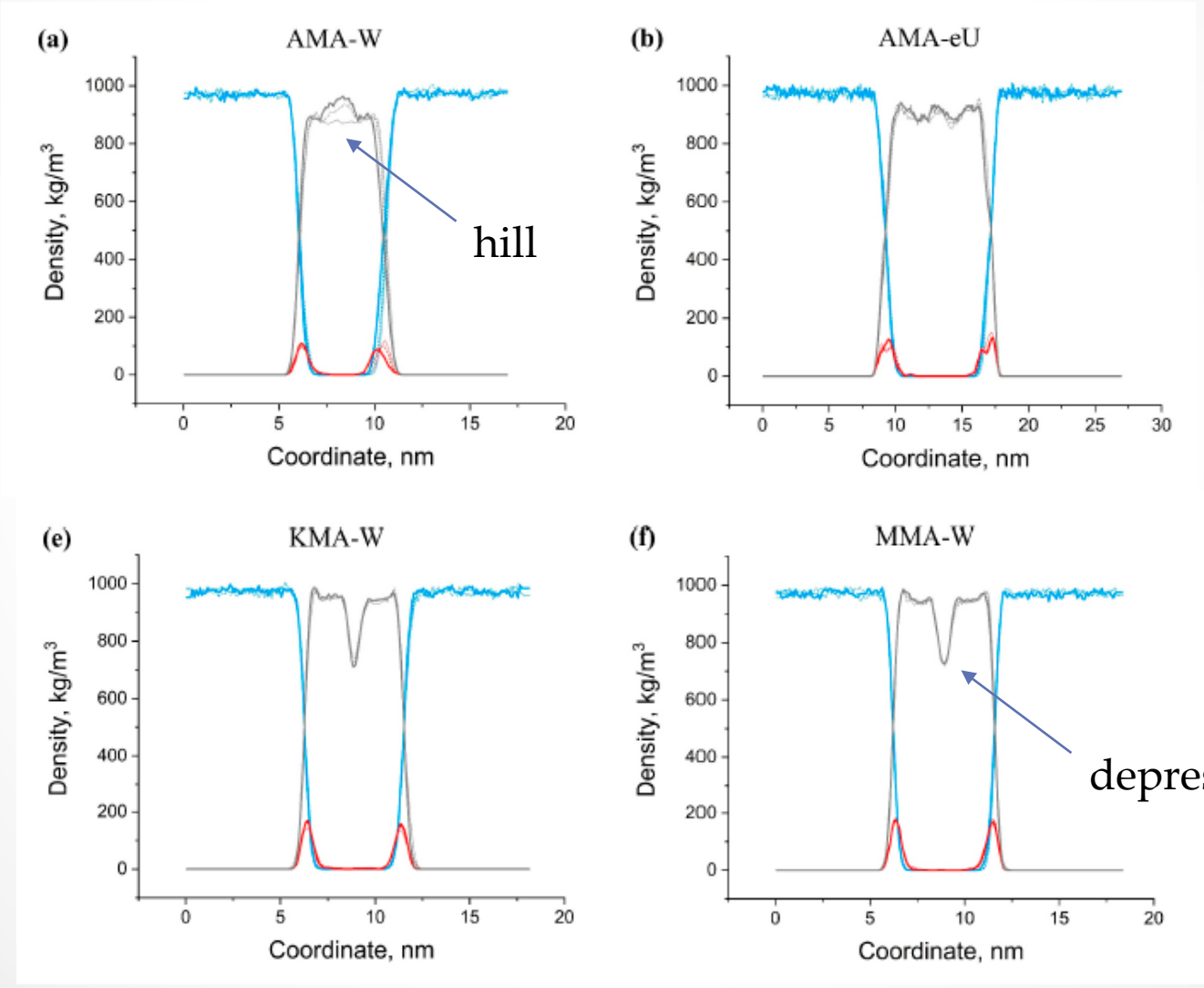
Membrane	Thickness, nm	Density, kg/m^3	Surface Density, Molecules/nm^2
AMA-W	4.4	857.5	2.16
AMA-W (1200 ns)	4.4	862.7	2.16
AMA-eU	7.8	907.3	3.84
AMA-sZ	5.9	891.5	2.93
AMA-(sZ+eU)	5.6	865.6	2.69
KMA-W	5.1	901.3	2.31
MMA-W	5.3	893.9	2.40
140eU–30W–30W [1]	5.9	898.2	2.86
140W–30W–30W [1]	4.6	861.8	2.17
100eU–50W–50W [1]	5.0	876.8	2.38
100W–50W–50W [1]	4.5	865.6	2.12

[1] *n*X–*m*W–*m*W: bilayer membranes constructed from *n* AMA-X (X = W or eU), *m* KMA-W, and *m* MMA-W molecules (equal numbers of all conformations in each leaflet).

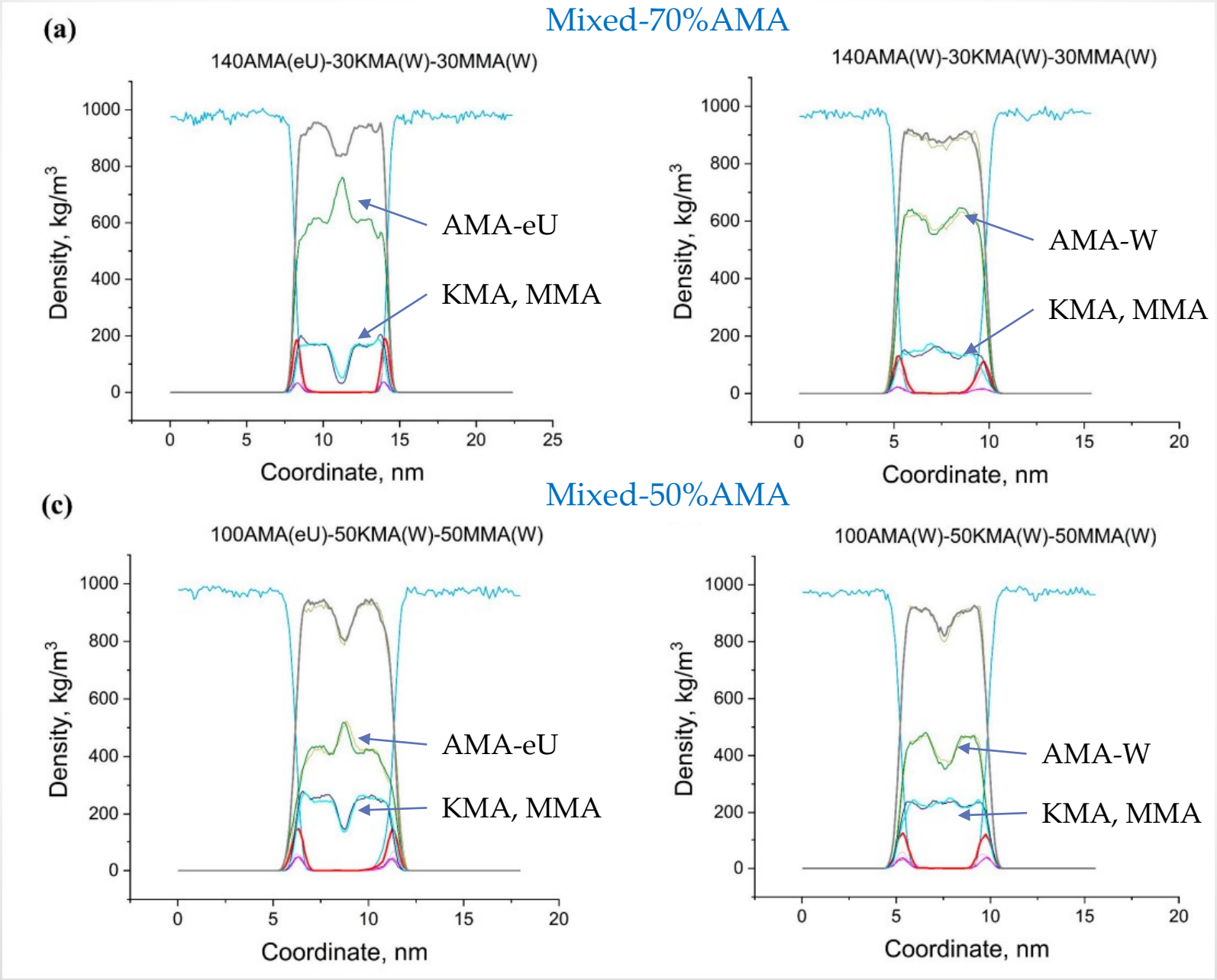
The membrane with AMA in the eU conformation is much thicker and, at the same time, much denser. This promotes the formation of a much stronger cell wall that should be much more resistant to the threatening external factors.

The composition of the membrane determines the its density profiles (the mass distribution along the cross section)

Single-component membranes



Density profiles - multicomponent membranes: KMA, MMA are concentrated at surface



Conclusions:

- Structure and properties of the bilayer MA membranes strongly depend on their initial packing and the presence of the KMA and MMA components.
- For the AMA-based membranes, the most labile conformation is W, which changes significantly within 300 ns to an extent of 50-70%.
- The conformational transitions in the AMA-W membranes are described by the first-order kinetics with characteristic times of 160–220 ns.
- In contrast to AMA, the KMA and MMA single-component W-membranes are very slowly transformed into other forms, due to the existence of hydrophilic groups at point *d*.
- In the multicomponent membranes, KMA and MMA are in the W conformation, they additionally stabilize conformations of AMA. Thus, KMA and MMA in the mycobacterial envelope play the role of a conformational stabilizer of AMA, increasing the stability of membranes.
- The membrane where AMA mostly has the eU conformation is much thicker and, at the same time, much denser. This creates much stronger cell wall that should be much more resistant to the threatening external factors.
- We conclude from the above observations that *MTB* can have a molecular mechanism that allows it to modulate the properties of the cell wall during its life using the regulation of KMA and MMA synthesis. The proteins regulating this synthesis can be a new target for the anti-tuberculosis drug design.

Further details:

Savintseva L.A.,… Ignatov S.K. et al. *Molecules*. 2023, **28**, 1347.
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