How does divalent ions content in the outer membrane of Gram-negative bacteria affect its properties?

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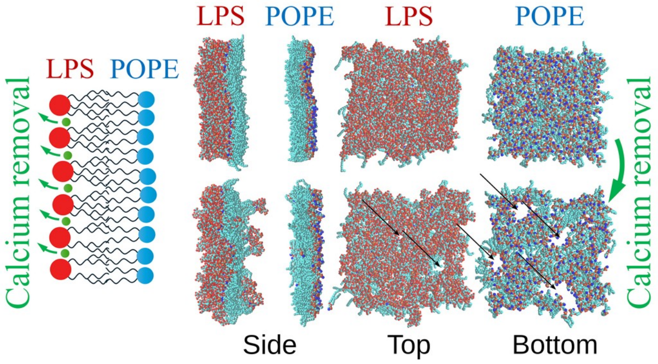

Figure 1. Illustration of the effect of a global stress on an asymmetric model of outer membrane of Gram-negative bacteria. The stress consists in the removal of all membrane-bound calcium ions and induces major membrane restructuring. For clarity, lipopolysaccharide (LPS) and phosphatidylethanolamine (POPE) components are presented separately in side and top views. Transient poration of the leaflets and positions of LPS flip-flooping are indicated by black arrows.

Over the past decades, polymyxins remain critical last-resort antibiotics against Gram-negative bacteria, despite their nephro- and neurotoxicity. However, the frequency of polymyxin-resistant bacterial strains is spiking, driving the need for alternative therapeutics eminently urgent.

One of the remarkable properties of polymyxins is their capacity to permeabilize the outer membrane of Gram-negative bacteria without utilizing protein channels. This membrane, characterized by its high rigidity due to the presence of lipopolysaccharides (LPS) in the outer leaflet, poses a formidable barrier to antibiotics. It is hypothesized that polymyxins disrupt this barrier by displacing divalent cations that bridge LPS molecules. Yet, the precise implications of this phenomenon on membrane structure and dynamics, as well as its significance in the mechanism of polymyxin action, remain a subject of debate.

We employed molecular dynamics simulations to model the effect of membrane-bound ions displacement. We investigated two non-resistant and two resistant models of the outer membrane of *Salmonella enterica*. We first focused on the effect of a large stress leading to the removal of all calcium ions, using atomistic simulations. We showed that this stress induces major membrane restructuring (Figure 1). Subsequently, we designed a collective variable (CV) that captures the local displacement of ions in a membrane. We applied enhanced sampling methods with this CV to estimate the impact of a local stress in the absence and presence of a polymyxin using both atomistic and coarse-grained simulations. Our findings offer an important insight in the consequences of membrane-bound divalent ions displacement, and help to seize its role in polymyxins mode of action.

Keywords: Gram-negative; bacteria; lipopolysaccharide; lipid; membrane; polymyxin; colistin; molecular dynamics