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Neutrophils Plasma Membrane Lipid Order Decreases and Is Required for Efficient Neutrophil Extracellular Trap Release

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Upon activation, neutrophils release chromatin extracellularly to fight pathogens in a process called NETosis. NETosis requires drastic membrane remodeling including plasma membrane (PM) microvesicle (MV) shedding, nuclear membrane (NM) remodeling and PM rupture. The biophysical mechanism of membrane remodeling during NETosis is unknown. Here, we used fluorescence lifetime imaging microscopy (FLIM) to assess the tension and order of the lipid bilayer during NETosis. We found that the lifetime of FlipTR - a lipid order and tension reporter - increases at the onset of NETosis, peaks at MV shedding, then decreases after NM remodeling. In contrast, the lifetime of Pro12A - a lipid order reporter - consistently decreases from the onset to the end of NETosis. This data suggests that early NETosis is dominated by increased tension in the lipid bilayer potentially due to decreased membrane reservoir following MV shedding, whereas later NETosis is characterized by membrane disordering. Our findings indicate that lipid order is tightly regulated during NETosis. Consistently, perturbing cellular cholesterol decreases NETosis efficiency, suggesting that membrane order changes, e.g. due to environmental conditions or diet, influence immune response. Identifying the associated biophysical mechanism will guide strategies that modulate lipid order to control NETosis for improved human health.