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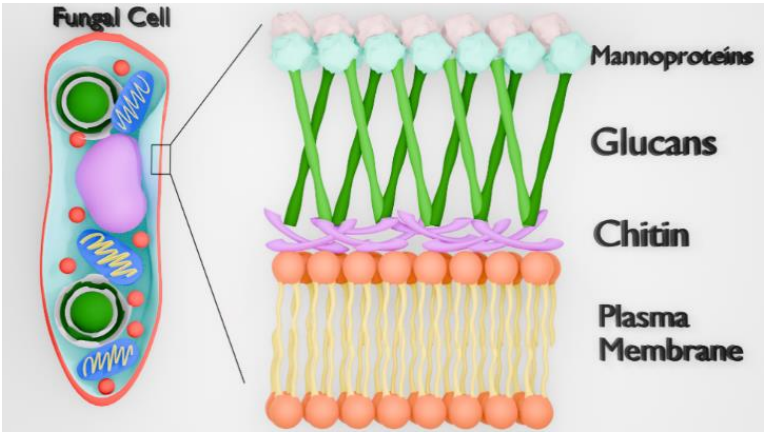
SONATA - Fungal membrane modeling for selected antifungal compounds interactions

Fungi are one of the most fascinating kingdoms of organisms on Earth. In the autumn, many of us head to the forest to collect beautiful specimens of mushrooms, which we then dry, pickle, or eat fresh. However, fungi also have a dark side, and it's not just about toadstools. Mushrooms also include various molds and fungal infections (commonly called mycoses), which do not inspire the same admiration as forest mushrooms. The problem of their presence is serious. In 2022, the World Health Organization published the first-ever list of priority fungal pathogens, identifying 19 fungi that pose the greatest threat to public health. Until now, one of the best protections for humans against fungi has been the difference in temperatures, as fungi could not function at our native temperature of 36.6 degrees Celsius. However, due to rising global temperatures and the incredible ability of fungi to adapt, this barrier is slowly disappearing. For example, a recent study in Chinese hospitals identified a previously unreported fungus in two patients. The pathogen was already resistant to the two most common antifungal drugs, and after exposure to higher temperatures, it quickly developed resistance, making it virtually untreatable with current methods. Cryptococcosis (fungal meningitis and encephalitis) is now one of the leading causes of death in sub-Saharan Africa. These and other reports highlight the urgent need to intensify research on fungal microorganisms and develop new antifungal agents.

There are many drug design strategies, but one of the most important aspects is identifying a molecular target, or the 'attack' site of a substance. One of the most promising targets is the cell membrane. In recent years, octenidine (an antibacterial substance) has achieved enormous commercial success by targeting the cell membrane, demonstrating the effectiveness of this approach. However, to apply this approach to fungi, we need a good model of the fungal membrane. Unfortunately, most research on fungi focuses on fungal mycelium, which is ideal for physiological and biochemical experiments but cannot be used to design drug action strategies.



The goal of this project is, first and foremost, to create a model of the cell membrane that could be used to design new antifungal drugs. Each species of fungus has a slightly different cell membrane structure, but a common denominator can be identified: various phospholipids, triglycerides, and ergosterol (the fungal equivalent of mammalian cholesterol). This means that we must create a model consisting of at least ten different types of molecules for it to accurately reflect the behavior of the cell membrane itself. For comparison, in bacteria, we have a much smaller diversity of lipids. For example, models of the *E. coli* membrane are characterized by only three different types of molecules.

Another very important aspect of building membrane models is taking asymmetry into account. Asymmetry is a key feature for cell function - every biological membrane is asymmetric - and its loss is an early sign of cell death. Asymmetry is a particularly important phenomenon in the case of fungal cells, which are often immobile and must achieve polarized growth to acquire nutrients, communicate with other cells, and reproduce. Only in recent years has there been technological development enabling the production of stable asymmetric membrane structures, including the use of microfluidic systems. Asymmetric membranes exhibit completely different dynamics and have completely different parameters than their symmetric counterparts, which shows the necessity of studying such models.

In this project, three models of fungal membranes will be created, which will then be subjected to the action of selected antifungal substances in order to study their mechanisms of action. These substances include, among others, the commonly used amphotericin B and Ciclopirox, suspected of interacting with ergosterol and altering the organization of the fungal membrane itself, as well as the novel experimental peptide RI18, which is believed to have the ability to mechanically damage the membrane. The result of this project will be obtaining a set of membrane parameters required for its proper functioning. Additionally, the effect of selected antifungal agents on its functioning will be assessed. This approach will not only reveal how these agents act on models of fungal membranes but will also allow for the identification of promising strategies for developing new antifungal agents that will directly target fungal membranes.