METHYLENE BLUE UPTAKE AND INTERMOLECULAR INTERACTIONS IN MICROBIAL CELLS THROUGH FLUORESCENCE-LIFETIME IMAGING MICROSCOPY (FLIM)

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Antimicrobial photodynamic therapy (APDT) is a promising tool to counterattack the emerging treat of drug-resistant pathogens. The technique combines low-intensity monochromatic light with a photosensitizer compound to produce reactive oxygen species (ROS) that can damage virtually any type of biomolecules and lead to rapid cell death. Since some ROS present diffusion-limited reactivity, most cell damage is co-localized with photosensitizer accumulation site. Hence, imaging photosensitizer accumulation and fluorescence lifetime in the nanoscale can bring a great level of information to further understand the ultrastructural cellular damage caused by APDT. In this study, we used a FLIM system capable of single-molecule detection to observe the accumulation and interaction sites of methylene blue (MB), a very broadly-used photosensitizer, in yeast, and Gram-positive and Gram-negative bacterial cells. Our data shows fluorescence lifetime contrast, with nanometric resolution, among different cellular structures such as cell wall, membrane and DNA. The images evidentiate differential MB accumulation in microbial cells and the existence of two different populations of MB molecular species: those interacting mostly with the solvent (short-lived, ~0.8 ns) and those interacting with biomolecules (long-lived, ~2 ns). The short-lived fluorescence predominates in the mucoid capsule of Gram-negative bacteria and cell-wall of yeast and Gram-positive bacteria while long-lived MB fluorescence shows preferential accumulation in DNA-rich sites[1]. It is marked in yeast nucleus and exclusively inside bacterial cells. In fact, literature supports that MB intercalation in nucleic acids stabilizes its excited-states leading to increased fluorescence lifetime and efficiency of singlet-oxygen production[2]. Our data brings evidence that this sort of phenomena can be observed by FLIM in the nanoscale and this should bring new insights to the photophysical, photochemical and biological mechanisms of photodynamic therapy.

References

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