Spatiotemporally Resolved Tracking of Bacterial Responses to ROS Mediated Damage at the Single-Cell Level with Quantitative Functional Microscopy

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Antimicrobial Photodynamic Inactivation (PDI) represents an attractive alternative in the treatment of infections by antibiotic-resistant pathogenic bacteria. In PDI a photosensitizer (PS) is administered to the site of the biological target in order to generate cytotoxic singlet oxygen ($^1O_2$) which reacts with the biological membrane upon application of harmless visible light. Established methods for testing the photoinduced cytotoxicity of PSs rely on the observation of the whole bacterial ensemble providing only a population-averaged information about the overall produced toxicity. However, for a deeper understanding of the processes that take place in PDI, new methods are required that provide simultaneous regulation of the ROS production, monitoring the subsequent damage induced in the bacteria cells, and full control of the distance between the bacteria and the center of the $^1O_2$ production. Herein we report on the implementation of photofunctional microparticles in combination with holographic optical tweezers (HOT) for the investigation of bacterial response to oxidative stress by means of quantitative functional fluorescence microscopy. A combination of a strongly hydrophobic axially substituted Si(IV) phthalocyanine adsorbed onto silica microparticles was developed, and the structural and photophysical characterization was carried out. The microparticles are able to produce reactive oxygen species (ROS) under the fluorescence microscope upon irradiation with visible light, and the behaviour of individual bacteria (*S. aureus* 6850) can be consequently investigated *in situ* and *in real time*. For this purpose, a methodology was introduced to monitor phototriggered changes with spatiotemporal resolution employing optical tweezers. The defined distance between the photoactive particles and individual bacteria can be fixed under the microscope before the photosensitization process, and the photoinduced damage is monitored by tracing the fluorescence turn-on of a suitable marker. The results showed a distance-dependent photoinduced death time defined as the onset of the incorporation of propidium iodide (PI). Our methodology constitutes a new tool for the *in vitro* design and analysis of photosensitizers for the treatment of cancer and infectious diseases with the aid of functional fluorescence microscopy, as it enables a quantitative response evaluation of living systems towards oxidative stress.

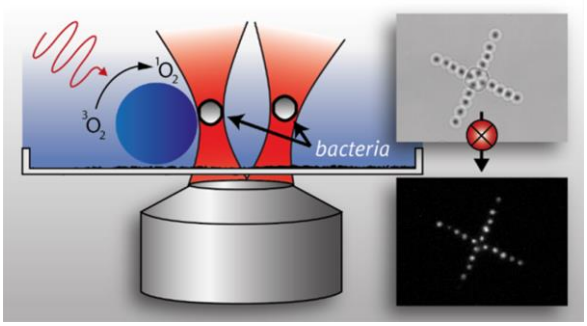

Figure 1. Graphical abstract of the optical fixation of *S. aureus* bacteria, the $^1O_2$ photoproduction on the functionalized microparticles and the PI signal increase of inactivated bacteria.

Work published as: A. Barroso[§], Malte Grüner[§], T. Forbes, C. Denz, C.A. Strassert, *„Spatiotemporally resolved tracking of bacterial responses to ROS-mediated damage at the single-cell level with quantitative functional microscopy"*, ACS Applied Materials and Interfaces, **2016**, 8, 15046-15057.