Site localisation on bacterial surface proteins using super-resolution imaging

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Abstract

Several significant pathogenic bacteria exhibit surface proteins that bind human proteins, such as antibodies, preventing them from being recognized by the human immune system. They are thus protected from elimination and can survive in the host organism. One common such bacteria is the *Streptococcus pyogenes* that exhibits the antibody binding M protein on its surface. *Streptococcus pyogenes* causes more than 700 million uncomplicated throat and skin infections annually and can be at root of rare but very serious invasive infections such as sepsis. Understanding the mechanism behind the binding tendencies of *Streptococcus pyogenes* may thus help explain what causes this significant difference in prevalence of infectious severity.

The aim of this study was to evaluate whether or not the super-resolution techniques stimulated emission depletion (STED) and stochastic optical reconstruction microscopy (STORM) are viable optical techniques for relative localization studies of bacteria-bound antibodies. Binding sites on bacterial surface proteins were localised using a previously known binding site on M protein. The bacterial membrane was adopted as a reference region.

The necessary resolution required for site localisation on M proteins was acquired by applying a method based on particle averaging and deconvolution on high resolution images. STED and STORM images were taken of samples prepared and analysed in the same manner. The two super-resolution techniques were compared to one another in terms of image quality, practicality and more specifically for the use of site localisation on bacterial surface proteins.

According to our results, STED together with a particle averaging method, may be an appropriate technique for relative site localisation studies on bacterial surface proteins. Our results also suggest that confocal microscopy together with an averaging method may be a reasonable alternative for relative site localisation. The results from the STORM images were inconclusive, suggesting that the STORM procedure may require more optimisation.