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Optimization of a nuclease-activated non-invasive optical imaging approach for *S. aureus* infections

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We previously described a novel molecular imaging technology capable of rapidly detecting *S. aureus* infections in mice. This was accomplished with an activatable near infrared (NIR) fluorescent probe consisting of a Cy5.5 fluorophore and a quencher attached to opposite ends of a synthetic oligonucleotide (RNA) sequence which is selectively cleaved by a secreted nuclease of *S. aureus*. Intravenous administration of this probe enabled the rapid detection of *S. aureus* infections in mouse thighs with non-invasive imaging. We are currently optimizing this approach for clinical applicability. This includes generating and testing probes with longer-wavelength fluorophores (i.e., that absorb and emit light of >750 nanometers). Near infrared wavelengths of >750 nanometers are known to penetrate tissues to a much greater extent than the ~700 nanometer light required to image the Cy5.5 fluorophore and could thus enable deeper imaging of tissues. However, the ability to robustly quench such "800 nm" fluorophores *in vivo* (a valuable endpoint for this optimization) has not been developed. Here, we will describe second generation quenched near-infrared fluorescent probes that include 800 nm fluorophores in place of Cy5.5. Quenching and nuclease-mediated activation were observed *in vitro* in buffer and in anticoagulated blood. Preliminary data indicate fluorescence increases at *S. aureus* infection sites and sites of nuclease injection in mice. Efforts to thoroughly characterize these probes *in vivo*, including in an *S. aureus* subcutaneous catheter biofilm infection model are ongoing.

Biography

Franklin Bright is currently pursuing his PhD in the Molecular and Cellular Biology at the University of Iowa, USA. He has received his Master's degree in Microbiology at Wagner College in Staten Island, NY, USA. Following his Master's research, he has worked as a Microbiologist carrying out antibiotic research at NovoBiotic Pharmaceuticals (Cambridge, MA, USA) for 5 years. While working at NovoBiotic, he engineered a strain library of over 4000 bacterial isolates.

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