Towards point-of-care detection of *Staphylococcus aureus* in synovial fluid using the RAPPID technology

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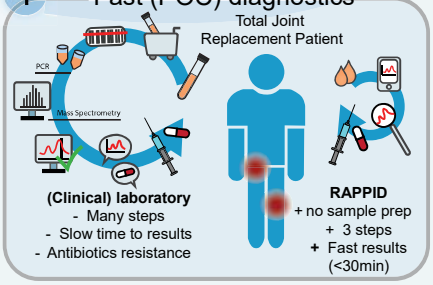
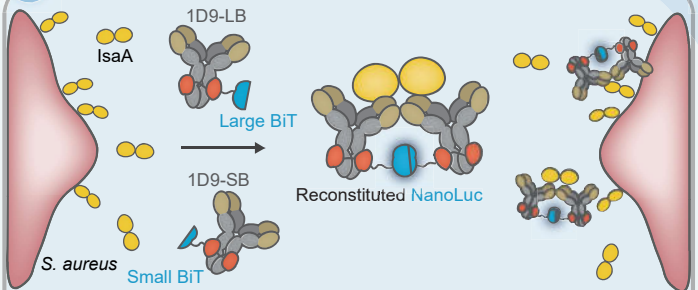
1 Fast (POC) diagnostics

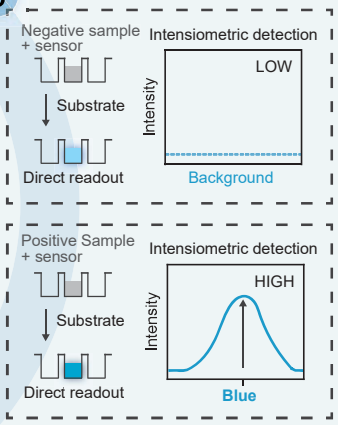


2 The IsaA RAPPID assay technology

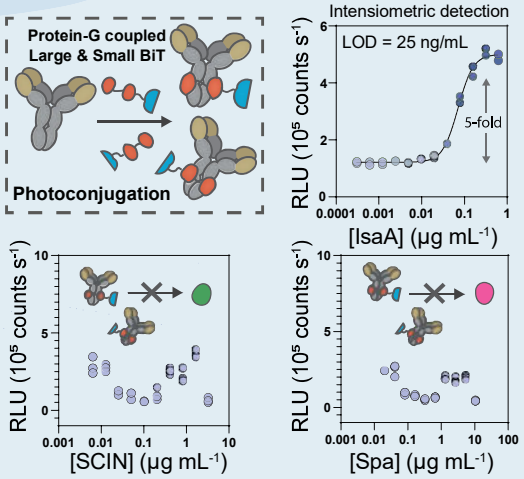


The concept of IsaA detection using the RAPPID technology. The primary antibody 1D9 is functionalized with a split version of the NanoLuc® luciferase (large BiT and small BiT) through the use of protein G-based photoconjugation. Antigen binding induces colocalization of the labelled antibodies and subsequent reconstitution of NanoLuc® resulting in the emission of blue light.

3

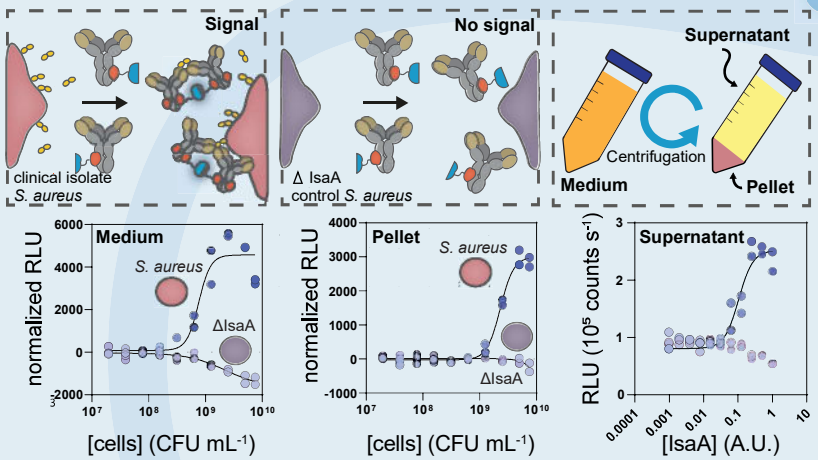


4 Succesful detection of IsaA in buffer



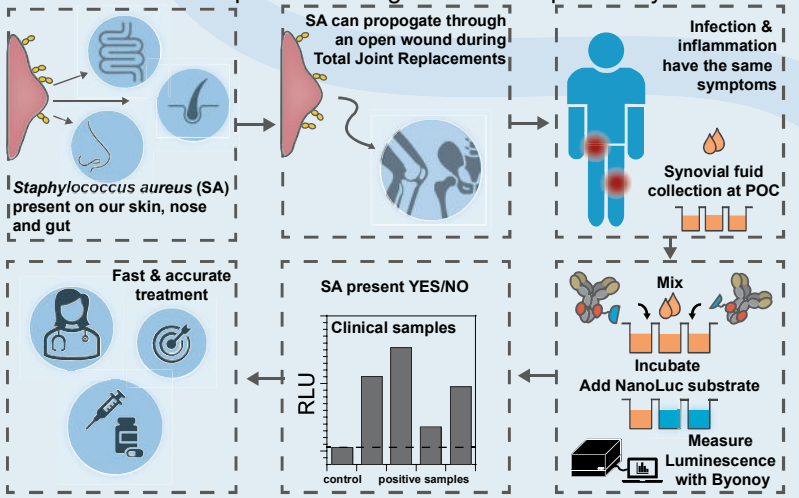
RAPPID sensor platform enables the detection of the immunodominant staphylococcal antigen A (IsaA), a key protein biomarker for identifying *S. aureus*-induced total joint replacement infections. Successful detection of IsaA was achieved with a limit of detection (LoD) of 25 ng/mL. Unlike the intensiometric detection of IsaA, other *S. aureus*-specific antigens showed no signal, highlighting the assay's high specificity alongside sensitivity.

5 Dual Detection of *S. aureus* and IsaA in Complex Media



With the working prototype, we proceeded to test cell samples under three conditions: cell pellet (*S. aureus* only), supernatant (secreted IsaA), and cell medium (*S. aureus* + IsaA). In all cases, both free IsaA and *S. aureus* were successfully detected, demonstrating the strong potential of the assay.

6 Towards clinical samples: Detecting *S. aureus* in patient Synovial Fluid



Staphylococcus aureus (SA) is commonly found on the nose, skin, and in the gut. During total joint replacement (TJR), SA can enter the body and cause infection. Because infection and inflammation show similar symptoms, they are difficult to distinguish. With the RAPPID test, synovial fluid (SF) is collected, mixed with sensor reagents, and measured. The resulting signal indicates whether infection-related biomarkers are present, allowing clinicians to make fast and targeted treatment decisions.

7 Conclusions IsaA-RAPPID immunoassay

- The **RAPPID sensor platform** enables **direct-from-sample, fast and specific** detection of the *S. aureus* antigen **IsaA**, a novel biomarker for TJR-related *S. aureus* infections.
- The **1D9 antibody**, functionalized with split NanoLuc fragments, allows **luminescence-based signal** generation upon IsaA binding.
- The assay shows **high specificity**, with no response to other *S. aureus*-specific antigens.
- Both **free IsaA** and **cell-associated IsaA** were successfully detected in different sample types (cell pellet, supernatant, and cell medium).
- These results demonstrate the **strong potential of RAPPID** for distinguishing *S. aureus*-related infection from inflammation in **synovial fluid samples**, enabling **rapid and targeted clinical decision-making** after TJR.

8 References

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