VISUALIZATION OF PENICILLIN-BINDING PROTEINS IN *STREPTOCOCCUS PNEUMONIAE* USING A VARIETY OF ACTIVITY-BASED PROBES

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Penicillin-binding proteins (PBPs) are membrane-associated proteins that are involved in the final stages of bacterial cell wall synthesis and are the target of β-lactam antibiotics. PBPs have received much attention for many decades as crucial antibacterial targets and for their role in antibiotic resistance. However, specific roles of individual family members in each bacterial strain are yet to be understood. Historically, the activity of the PBPs in membrane preparations was detected by radiolabeled penicillins in gel-based studies. Using this strategy, the relationship between the concentration of β-lactams and their selectivity for PBPs in various microorganisms was established. More recently, fluorescent β-lactams, such as Bocillin-FL (Boc-FL) replaced radioactive probes based on their many advantages including biocompatibility and application in live cell imaging.

We are interested in exploiting other available β-lactam antibiotics to make specific probes for individual PBPs in microorganisms such as *S. pneumoniae*. This is particularly critical for studying essential PBPs, which are indispensable and cannot be studied using other methods such as depletion. Along the same lines, we have developed a β-lactone scaffold that specifically targets individual PBPs in various microorganisms (**Figure 1**). Upon application of these probes to pneumococcal cells, the *in vivo* activity of PBP2x and 2b was successfully revealed for the first time. Extension of our probe library and its assessment in other microorganisms provides us with an invaluable toolbox to study PBPs as critical bacterial proteins.

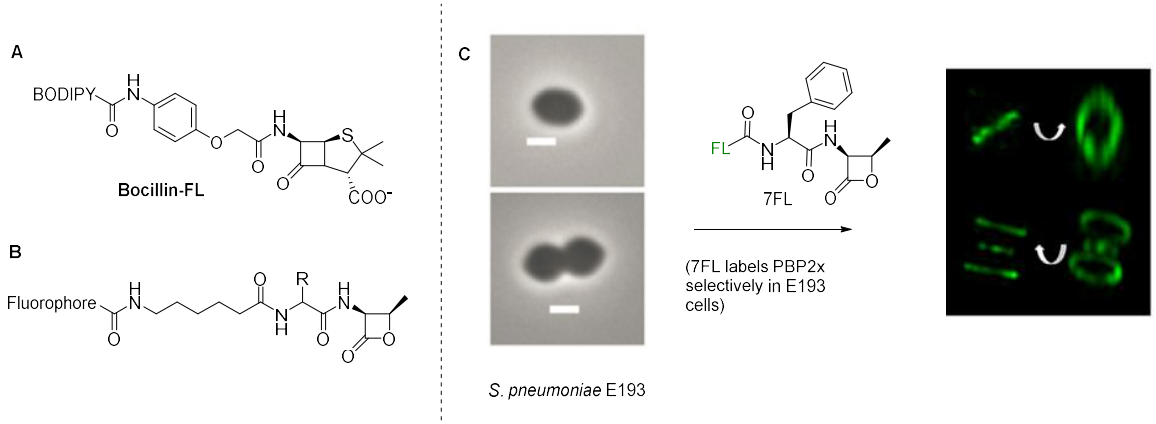

Figure 1. Visualization of PBP2x in *Streptococcus pneumoniae* cells. **A**) Structure of Bocillin-FL, **B**) General structure of β-lactone probes, **C**) *S. pneumoniae* Δ*pbp1b* mutant strain was labeled by our probe, 2R,3S-β-lactone-L-Phe-fluorescein (7FL), which is selective for PBP2x. High-resolution fluorescence microscopy image of labeled cells revealed septal activity of this PBP at mid- to late-divisional stage.