Supporting Information

Visualization and Quantification of Sortase Activity at the Single-molecule Level via Transpedidation-directed Intramolecular Förster Resonance Energy Transfer

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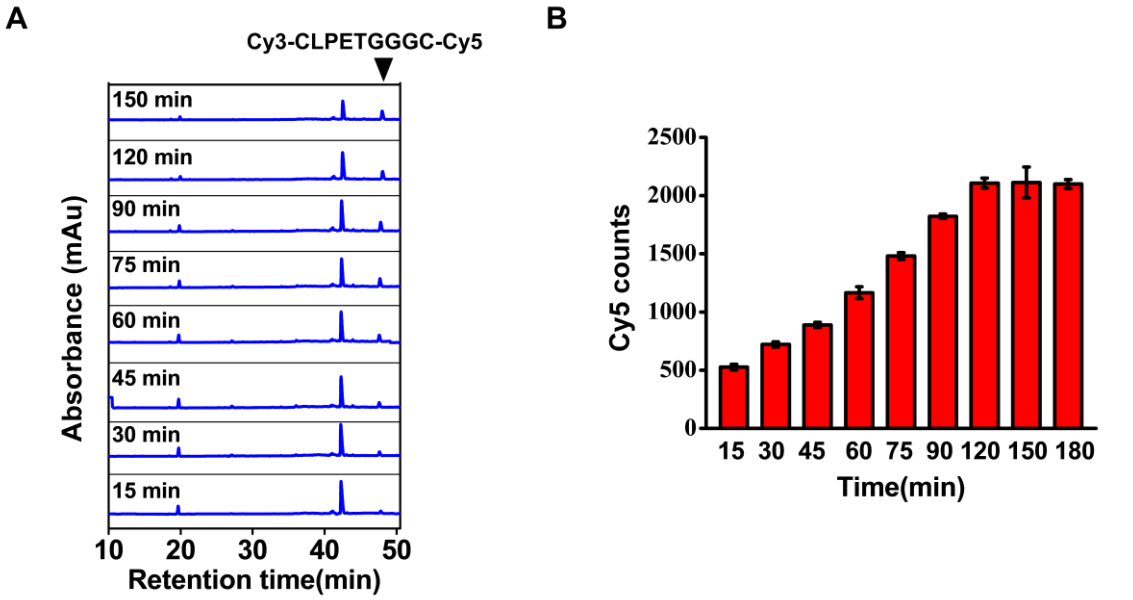

Figure S1. Time course of SrtA-catalyzed transpeptidation reaction. (A) HPLC analysis of SrtA-catalyzed ligation of Cy3-CLPETGG with GGGC-Cy5 to form Cy3-CLPETGGGC-Cy5 at different time points. The signal was detected under UV_{220}. The peak that corresponds to the Cy3-CLPETGGGC-Cy5 was indicated by inverted triangle. (B) Variance of Cy5 counts with reaction time. The concentration of SrtA is 20 nM. Error bars show the standard deviation of three independent experiments.

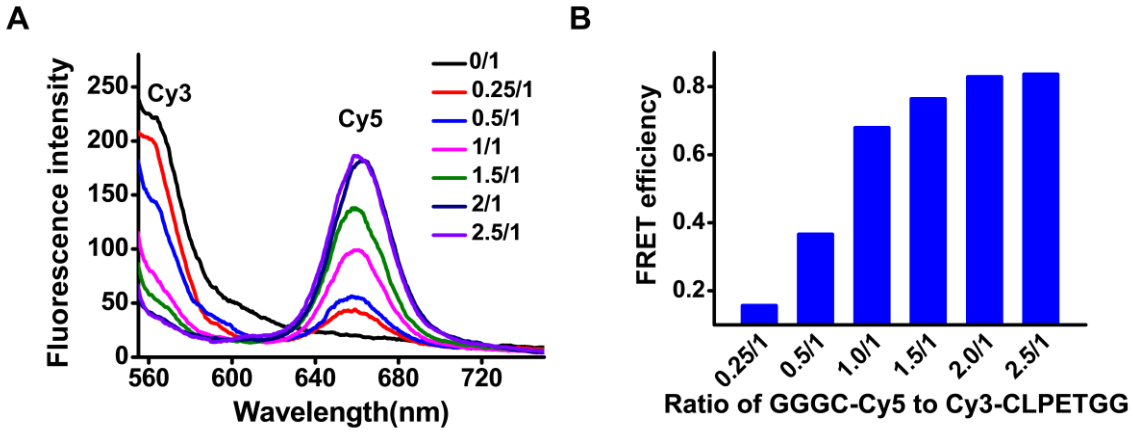

Figure S2. (A) Fluorescence emission spectra of Cy3 and Cy5 in response to different ratios of GGGC-Cy5 to Cy3-CLPETGG (λ_{ex} = 520 nm). (B) Variance of FRET efficiency with different ratios of GGGC-Cy5 to Cy3-CLPETGG.

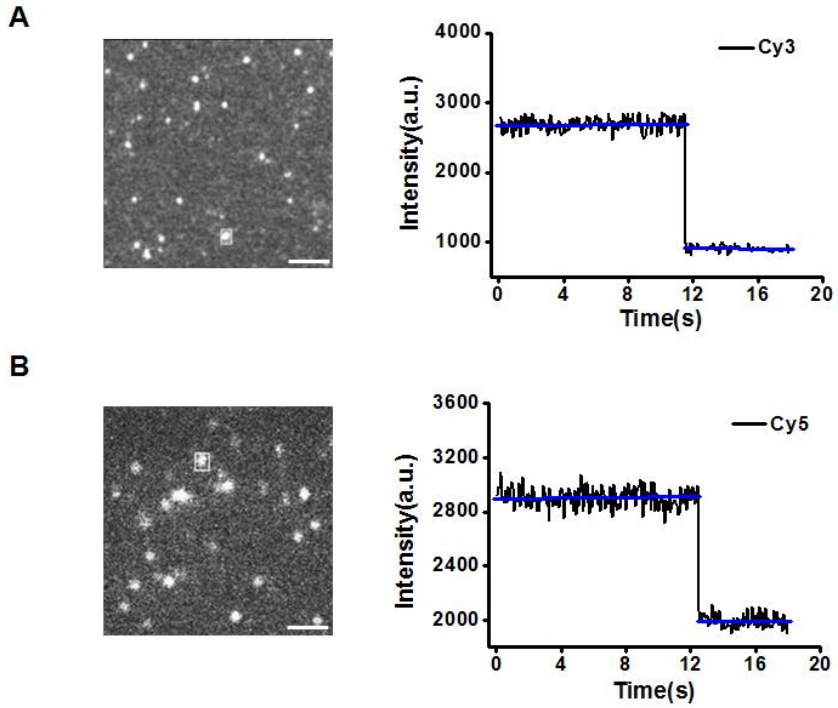

Figure S3. Examples of intensity traces over time showing one photobleaching step for individual Cy3 (A) and Cy5 (B) fluorescence spots. The fluorescence images prior to photobleaching process are shown in the left panel. Scale bar is 5 μm.

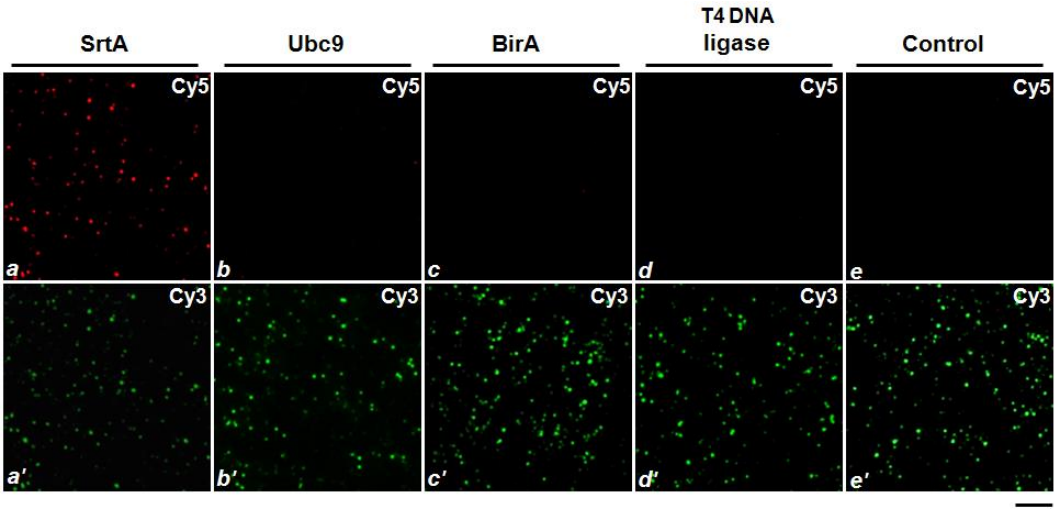

Figure S4. Fluorescence images of Cy3-CLPETGG/GGGC-Cy5 mixture following incubation with SrtA (*a*, *a*'), Ubc9 (*b*, *b*'), BirA (*c*, *c*'), and T4 DNA ligase (*d*, *d*'), respectively. The mixture without any treatment (*e*, *e*') was used as the control. The red color represents the signal of Cy5, and the green color represents the signal of Cy3. Scale bar is 5 μm.

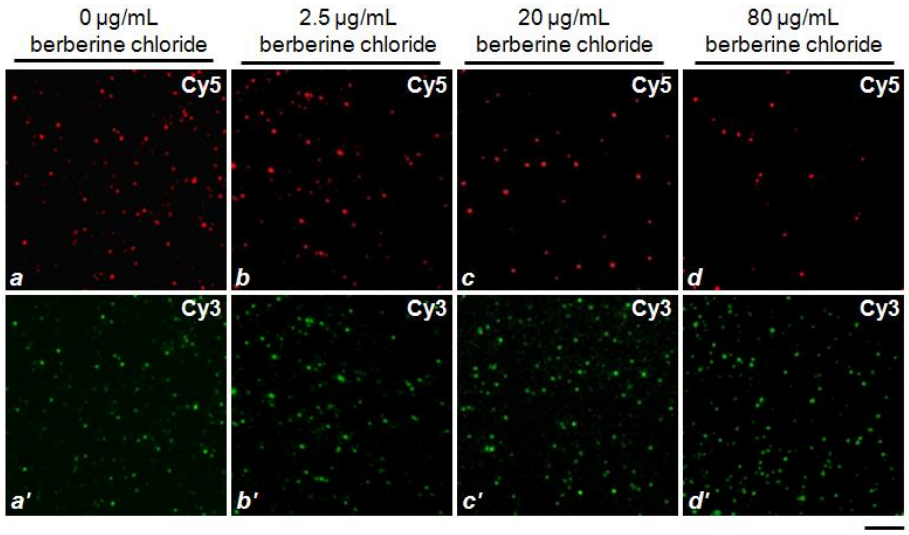

Figure S5. Fluorescence images of Cy3-CLPETGG/GGGC-Cy5 mixture following incubation with berberine chloride adminstrated to *S.aueus* cells. The *S.aueus* cells were pre-treated with 2.5 μg/mL (*b*, *b'*), 20 μg/mL (*c*, *c'*), and 80 μg/mL (*d*, *d'*) berberine chloride, respectively, to inactive intracellular SrtA. The cells without berberine chloride treatment (*a*, *a'*) was used as a control. The red color represents the signal of Cy5, and the green color represents the signal of Cy3. Scale bar is 5 μm.

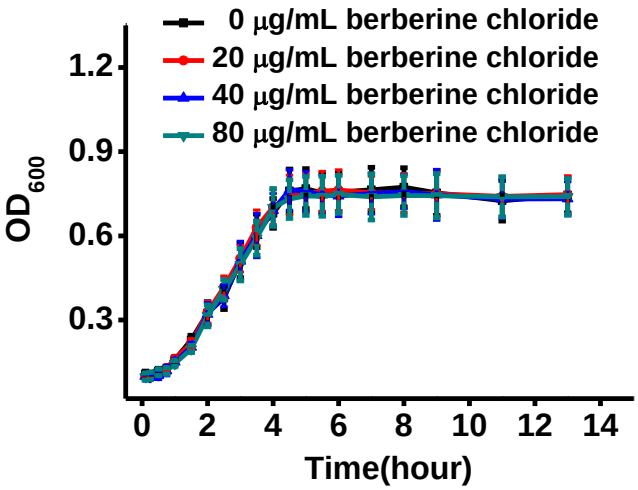

Figure S6. The growth curve of *S.aueus* cells following treatment with different-concentration berberine chloride. The samples were taken at the indicated time points to measure the absorbance at 600 nm. Error bars show the standard deviation of three independent experiments.