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Transpeptidation-Mediated Assembly of Tripartite Split GFP for Label-Free Assay of Sortase Activity

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Abstract

Transpeptidation of surface proteins catalyzed by the transpeptidase sortase plays a critical role in the infection process of gram-positive pathogen, and probing sortase activity and screening its inhibitors are of great significance to fundamental biological research and pharmaceutical development, especially novel anti-virulence drug design. Herein, we developed a novel fluorescent biosensor to detect sortase activity based on a transpeptidation-triggered assembly of tripartite split green fluorescent protein (split GFP). Peptide P_1, composed the 10^{th} β-sheet of GFP (GFP10) and the sortase A (SrtA) recognition sequence (LPETX), and peptide P_2, the 11^{th} β-sheet of GFP (GFP11) with oligoglycine at N-terminal, were designed and synthesized, respectively. Existence of SrtA enables P_1 and P_2 to ligate into one peptide, which could spontaneously bind to GFP1-9 (the 1^{st}–9^{th} β-sheets of GFP) and assemble into functional GFP. Thus, the sortase-catalyzed transpeptidation can switch on the fluorescence signal of GFP. The method was successfully applied to detect SrtA activity with a low detection limit of 0.16 nM, and for its inhibition measurement. Moreover, the feasibility of the proposed assay was further expanded to detect SrtA in human blood, and further gram-positive pathogens analysis in frozen food. Our method, using tripartite split GFP as a readout, is facile, label-free and sensitive, and exhibits great potential as a promising platform for sortase detection and inhibitor screening.

Introduction

Gram-positive pathogenic bacteria, such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis*, cause a wide spectrum of diseases ranging from minor skin infections and soft tissue infections to osteomyelitis, meningitis, endocarditis, septicemia, and toxic shock syndrome.[1-3] Sortase, a group of transpeptidases, plays a critical role in the different stage of the pathogenic process through catalyzing the attachment of the clear majority of surface proteins to bacterial cell wall. Sortase recognizes and cleavages a carboxyl-terminal sorting signal (LPETG motif) between threonine (T) and glycine (G) residues to form an acyl-enzyme intermediate. It then catalyzes the formation of an amide bond between the carboxyl group of the T and the cell wall precursor molecule lipid II, creating the LPETG-containing proteins that can promote bacteria adhere to host cells and tissues.[4-10] Nowadays, drug resistance of pathogens becomes a world-wide health problem, due to excessive use and abuse of antibiotics.[11] As a virulence-related molecule, sortase is not necessary for bacterial growth, and its inhibitors exert little selective pressure on pathogens to develop drug resistance.[12] Consequently, sortase is considered as an ideal target for novel anti-virulence drug design and development, and has attracted a lot of interest. Probing sortase activity and screening its inhibitors are of great significance to not only fundamental biological research, but also pharmaceutical development and medical diagnostics.[13] Currently available methods, such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS),[14-19] could quantitatively detect sortase activity by directly monitoring its

catalytic reaction products, but suffer from complex processes and special technique requirements. Comparatively, fluorescent enzyme analysis has some inherently advantages, including high sensitivity, simplicity and high-throughput capability. For efficient signal generation, the synthetic substrate peptides labeled with fluorophore or quencher were generally exploited as probes in fluorescent transpeptidase assays, and the catalytic efficiency was judged by fluorescence quenching or fluorescence resonance energy transfer (FRET).[20,21] Such assays are effective but with the drawback of low signal-to-background ratio. Thus, it is highly desirable to develop new strategies for convenient, cost-effective and label-free detection of sortase activity.

Green fluorescent protein (GFP) is widely recognized as a powerful molecular tool in modern life sciences because of its intrinsic fluorescence and genetically encoded properties.[22-24] Based on reconstitution of fragments of GFP and its variants split into two pieces, split GFP technique was developed to evaluate protein-protein interactions, protein folding and solubility, for fluorescent labeling of intracellular and cell surface proteins, and also for nucleic acids detection.[25-28] Inspired by these studies, our group developed a method for label-free detection of protein kinase activity on the basis of phosphorylation-mediated assembly of a semisynthetic fluorescent protein.[29] Recently, Waldo group[30] reported a tripartite split GFP, which consists of two small peptides GFP10 and GFP11, and a third large GFP1-9 fragment. These two small sized peptides can be artificial synthesized, endowing the newly described tripartite split GFP system with great design versatility for different kinds of assay, while only a

protein-protein association sensor has been developed so far.

Herein, we presented a facile and label-free method for sortase activity assay based on the tripartite split GFP assembly system. In this work, sortase A (SrtA), a kind of transpeptidase prevalent in gram-positive bacteria,[31,32] was chosen as a model. Two peptide probes P_1 and P_2 were rationally designed by coupling the sorting sequence (LPETX, X=Glycolic acid-G-OH) of SrtA to the C-terminal of GFP10 (P_1, GFP10-LPETX) and polyglycine (GG) to the N-terminal of GFP11 (P_2, GG-GFP11), respectively. Transpeptidation catalyzed by SrtA resulted in the ligation between P_1 and P_2, which subsequently assembled with GFP1-9 to form intact GFP with fluorescence. This transpeptidation-mediated assembly of tripartite split GFP enables facile, switch-on and quantitative analysis of SrtA activity without peptide labeling. Since the synthetic peptide probes can be easily replaced by the substrate of other transpeptidases, this tripartite split GFP-based method has the potential to be a versatile platform for transpeptidase assays and inhibitor screening.

Experimental Section

Materials and Measurements.

Peptides GFP10-11 (DNHYLSTQTVLSKDPNEKRDHMVLHESVNAAGIT, the 10^{th}-11^{th} β-sheet sequences of GFP), GFP10 (DNHYLSTQTILLKDLN, the 10^{th} β-sheet sequence of GFP), GFP11 (RDHMVLLEYVTAAGIT, the 11^{th} β-sheet sequence of GFP), P_1 (DNHYLSTQTILLKDLN-LPET-Glycolic acid-G-OH), P_2 (GG-RGDRDHMVLLEYVTAAGIT), P_F (RE(Edans)CCLPATGR), and P_Q

(EEEEEEEEEY(PO_3)RRGGGGK(dabcyl)RGRKKrRQRrR) were synthesized by KareBay™ BioChem (Ningbo, China). Quercetin and curcumin were bought from Solarbio Life Sciences (Beijing, China). All other chemicals were of analytical grade. All solutions were prepared using ultrapure water (18.2 MΩ·cm) from Millipore (Milli-Q) system.

The UV-vis absorption spectra were recorded on a Cary60 UV-vis spectrophotometer (Agilent, USA). The fluorescence measurements were performed on SynergyMx Microplate reader (BioTek, USA), and recorded three times for each sample (n = 3). The results show the average of the measurements with error bars indicating the relative standard deviation. The peptide molecular was determined using a MALDI-TOF mass spectrometry (Bruker, USA). Cell breaking was performed on a SCIENTZ-IID ultrasonic cell disruption system (Ningbo, China).

Protein Expression and Purification.

The amino acid sequences of GFP1-9 and sortase A (SrtA) were referred to the reported literature.[30,33] The gene sequence encoding was reversely translated from the amino acid sequence and optimized for *E. coli* codon usage. The full-length gene, which is inserted in plasmid pET28 (SrtA) and pCold (GFP1-9), respectively, were synthesized by Sangon (Shanghai, China). The plasmid pET28-srtA and pCold-gfp1-9 were transformed into *E. coli* BL21 (DE3) by thermal conversion, respectively. Cells were grown in LB medium at 37 °C until OD_{600} (optical density at 600 nm) reached about 0.6, and then were induced by isopropyl β-D-1-thiogalactopyranoside (IPTG).

The culture was collected by centrifugation, and washed 3 times in buffer solution. The resultant cell suspension was broken using a SCIENTZ-IID ultrasonic cell disruption system. After centrifugation at 8000 rpm for 15 min, the clarified supernatant was purified by Ni-NTA agarose chromatography, and desalted by desalination chromatography (ÄKTA, GE). SrtA and GFP1-9 were investigated by SDS-PAGE assay. The purified GFP1-9 and SrtA were qualified by the improved Bradford protein assay dye reagent kit with bovine serum albumin (BSA) as the standard and stored at -20 °C.

Split GFP Assembly and SrtA-induced Ligation.

For split GFP assembly, GFP1-9 (1 μM) and GFP10-11 (0-4 μM) were mixed at different ratios in Tris-HCl buffer (10 mM Tris, 100 mM NaCl and pH 7.4) in a final volume of 100 μL, and incubated at room temperature for 2 h. Then fluorescence and absorption spectra of the solution were recorded.

To validate the SrtA catalyzed ligation, SrtA was mixed with peptide P_F and P_Q in SrtA reaction buffer (50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, and pH 7.5) at 25 °C for 2 h, and then the fluorescence intensity was recorded. Peptide P_1 (20 μM) and P_2 (160 μM) was treated with SrtA (200 nM) in SrtA reaction buffer at 25 °C for 2 h. The resulting solution was centrifugated and filtered at 10000 rpm for 30 min by 3K Ultrafiltration centrifuge tube (Millipore). Then the reaction solution was measured by the MALDI-TOF MS.

Detection of SrtA Activity and Inhibitor Screening.

For SrtA detection, the fluorescence signals toward different concentrations of SrtA were measured under the optimized conditions. GFP1-9 (2 μM), P_1 (5 μM) and P_2 (40 μM) were mixed in SrtA reaction buffer (50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, and pH 7.5). Then SrtA at different concentrations were added to a final volume of 100 μL, respectively, and incubated at 25 °C for 3 h. Fluorescence spectra of each sample were recorded on a Microplate reader (BioTek, USA). The excitation wavelength was 480 nm, and the emission wavelength was in the range from 500 to 600 nm with both excitation and emission slits of 9 nm.

For SrtA inhibition assay, different concentrations of quercetin (0-300 μM) or curcumin (0-700 μM) were mixed with SrtA (50 nM), P_1 (5 μM), P_2 (40 μM) and GFP1-9 (2 μM) in SrtA reaction buffer to a final volume of 100 μL and incubated at 25 °C for 3 h. Fluorescence spectra of the samples were recorded on the Microplate reader.

The human serum samples were prepared by centrifuged human blood 5 min at 10000 rpm and filtered by 5K Ultrafiltration tube (Millipore) at 12000 rpm for 15 min. Then 2.5 μL SrtA at different concentrations (0.4, 2.0 and 4 μM) were added into 77.3 μL human serum, respectively, and subsequently the analysis solution (mixture of 0.5 μL 1 mM P_1, 4.0 μL 1 mM P_2, 15.7 μL 12.7 μM GFP1-9) was added to a final volume of 100 μL, and incubated at 25 °C for 3 h. Fluorescence spectra of the samples were recorded on the Microplate reader.

Bacteria Detection.

Staphylococcus aureus (*S. aureus*) and *Escherichia coli* (*E. coli*) were grown in LB medium at 37 ℃ with shaking at 200 rpm or plated on LB agar (*S.aureus* plate on Baird-Parker plate), respectively. The bacteria concentrations were calculated by plate counting method.[34,35] Different concentrations of bacteria (1-10^7 CFU mL^{-1}) were added into a 96-well plate, and then P_1 (5 μM), P_2 (40 μM), and GFP1-9 (2 μM) were added, respectively, with a final volume of 100 μL, and incubated at 25 ℃ for 3 h. The fluorescent intensity was recorded on the Microplate reader.

Frozen foods including dumplings and glue pudding, and pasteurized milk were chosen as the samples to evaluate the number of *S. aureus* in the real application assay. For dumplings and glue pudding, the samples were collected, crushed into powder, and weighed (0.75 g) into 10 mL LB medium, respectively, as well as 20 μL pasteurized milk, and then placed at 37 ℃ overnight. Then the bacterium analysis in complex samples was simply measured by mixing the cultures (20 μL) with P_1 (5 μM), P_2 (40 μM), and GFP1-9 (2 μM) solution in a final volume of 100 μL at 25 ℃ for 6 h. Meanwhile, 20 μL cultures were taken and coated on Baird-Parker plating for plate count after incubation overnight.

Results and Discussion

Principle of SrtA Activity Detection Based on Tripartite Split GFP Assembly.

Firstly, whether GFP1-9 and the synthetic peptide (GFP10-11) could spontaneously assemble to form intact functional GFP was investigated. The large fragment GFP1-9 and SrtA were expressed and purified according to the previously reported

literature,[30,33,36,37] and identified by SDS-PAGE (Figure S1). The relative fluorescence intensity, defined as F/F_0, was used to evaluate the fluorescence recovery efficiency, where F referred to the fluorescence intensity of the assembled GFP, and F_0 referred to that of GFP1-9 alone. Figure 1A presents the fluorescent analysis of GFP fragments assembly. The solo GFP1-9 had no obvious fluorescence at 510 nm (column 1), because of the chromophore deficiency, and there was also no fluorescence recovery when the two single short peptides GFP10 and GFP11 were added (column 2). However, the addition of GFP10-11 induced a significant enhancement of GFP1-9 fluorescence (F/F_0=144.0, column 5), which can be attributed to the spontaneously assembly of GFP1-9 and GFP10-11 and then the fluorescence recovery of GFP. In addition, the ratio of GFP10-11 to GFP1-9 was also optimized. As shown in Figure S2, the relative fluorescence intensity increases with the increase of the ratio of GFP10-11 to GFP1-9, and then reaches a relatively stable platform when the ratio is 2:1. Besides, a dissociation constant (K_d) of 157.9 ± 2.38 pM was calculated (described in the SI[38]) according to the relative fluorescence intensity plot (Figure S2B), which indicates the high affinity between GFP10-11 and GFP1-9 and consequently their highly efficient assembly.

Secondly, we examined whether the expressed SrtA was catalytically active as expected. According to the previously reported method,[39] its transpeptidase activity can be detected by using fluorophore and quencher-modified peptide probes (P_F and P_Q). As shown in Figure S3, after P_F and P_Q were mixed with SrtA, the fluorescence intensity of the solution was quenched by 27.2% compared to that of without SrtA.

Such fluorescence quenching suggested that SrtA can catalyze the ligation between P_F and P_Q, and the closed distance lead to the quenching. Then, two peptide probes P_1 and P_2 were rationally designed by coupling the sorting sequence (LPETX) of SrtA to the C-terminal of GFP10 (P_1, GFP10-LPETX) and polyglycine (GG) to the N-terminal of GFP11 (P_2, GG-GFP11), respectively. The product of P_1/P_2 ligation by SrtA was analyzed by MS, and a new peptide with molecular weight of 4.528 kDa was detected (Figure 1B), which is in accord with that of P_1-P_2 (GFP10-LPETGG-GFP11) peptide. Collectively, these data indicated that the expressed SrtA is transpeptidase active, and can efficiently catalyze the ligation between the probes P_1 and P_2.

Next, several experiments were carried out to demonstrate the assembly of GFP1-9 and the transpeptidation product P_1-P_2. Non-denaturing polyacrylamide gel (native-PAGE) showed that when P_1-P_2 was mixed with GFP1-9, a new band with a similar molecular weight of the GFP1-9/GFP10-11 complex was detected, which emitted bright green fluorescence under ultraviolet lamp (Figure 1C). In addition, when GFP1-9 was mixed with P_1-P_2, the typical absorption and excitation spectra of GFP (Figure S4 and S5), and further a strong fluorescent signal were observed (Figure 1A, column 4), suggesting that P_1-P_2 can assemble with GFP1-9 and then form intact GFP. While the separated probes, P_1 and P_2, cannot interact with GFP1-9 (Figure 1A, column 3). Furthermore, after the binding stoichiometry of P_2 to P_1 was optimized to 8:1 (Figure S6), a relative fluorescence intensity of 120.8 was achieved, and the

assembly efficiency was calculated up to 83.9% when compared to that of the GFP1-9/GFP10-11 complex.

Integrating the SrtA-mediated transpeptidation with the assembly of GFP1-9 and GFP10-11/P_1-P_2, we proposed a novel tripartite split GFP assembly platform for label-free detection of sortase activity. As described in Scheme 1, SrtA recognizes and cleavages the LPETX motif in P_1, and then catalyzes the covalent linkage between the peptide P_1 and P_2. The resulting peptide P_1-P_2 can spontaneous assembly with GFP1-9 to form intact GFP with fluorescence emission. Therefore, the fluorescence intensity can respond to SrtA activity.

SrtA Sensing Based on Tripartite Split GFP Assembly.

To obtain a better sensing performance, some critical issues were taken into account. The SrtA-catalyzed reaction is reversible when using the LPETG pentapeptide as recognition sequence.[40] However, when a depsipeptide substrate (LPET-Glycolic acid-G-OH) was used, the conversion efficiency can reach up to 87%, because the hydroxyacetyl byproduct prohibits the reverse reaction.[41] Herein, the depsipetide substrate was used as the sorting motif of SrtA in this work. Besides, considering the the two-step reaction is complex and time-consuming, we wondered whether split GFP could also assemble well in SrtA reaction buffer. Although there is difference between SrtA reaction buffer (50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, and pH 7.5) and split GFP assembly reaction buffer (10 mM Tris, 100 mM NaCl and pH 7.4), the comparable fluorescence recoveries in both the buffers were detected (Figure S7).

Thus, the experiment procedure was simplified from two-step reaction to one-pot reaction in the following experiments. Further, the reaction time and temperature were studied in detail, and incubation at 25 ℃ for 3 h (Figures S8 and S9) was used for the subsequent SrtA activity assay and inhibitor screening.

Under the optimized condition, SrtA activity measurement was carried out. Figure 2A demonstrated the fluorescence responses of the assay to SrtA at different concentrations. The fluorescence intensity increased along with the increasing concentration of SrtA ranging from 0.3 to 500 nM. The relative fluorescence intensity exhibited a good linear correlation ($R^2 = 0.993$) to the SrtA concentration in the range of 0.3-100 nM (inset in Figure 2B) with a low limit of detection (LOD = 0.16 nM). The LOD of the proposed method is much lower than that of the other methods (355 nM[14] and 100 nM[19]), because of the high affinity of P_1-P_2 with GFP1-9 and the low background of the non-fluorescent GFP fragments (GFP1-9). Moreover, simply by mix-and-readout procedure, the activity of SrtA can be sensitively and directly detected by the proposed fluorescence homogeneous method.

SrtA Inhibition Assay.

Since sortase is considered as an ideal target for novel anti-virulence drug design and development, sortase inhibition assay, as well as sortase activity assay, is very important for drug screening.[12] Here, two potent inhibitors (quercetin and curcumin[42]) of SrtA were chosen to investigate whether the method can be used for SrtA inhibitor screening. The inhibition efficiency (IE) was determined by the following equation,

$$IE(\%) = 100 \times (F - F_i)/(F - F_0) \quad (1)$$

where F_i is the fluorescence intensity of GFP1-9 with P_1 and P_2 in the presence of both SrtA and its inhibitor. As shown in Figure 3A, 3B, the inhibition efficiency was found to increase along with increasing inhibitor concentration, indicating more quercetin or curcumin results in more SrtA inhibition and less recovery of fluorescence. Sigmoidal fitting was applied to plot the inhibition curve, and IC_{50} values of quercetin and curcumin were calculated to be 7.8 μM and 75.4 μM, respectively (insets in Figures 3A and 3B), which were comparable to those reported in the literatures.[43,44] Both IC_{50} values of quercetin and curcumin are far lower than the minimal inhibitory concentration values (MIC) against *Staphylococcus aureus* (*S. aureus*, 900 μM[45] and 200 μM[46], respectively), which means that the two potential SrtA inhibitors, especially quercetin, have negligible influence on bacterial viability at low concentration, but can efficiently inhibit the activity of SrtA, and further decrease bacterial virulence. Thus, SrtA inhibitors have the potential to become anti-infection drugs and hold great promise for the anti-virulence therapies. Furthermore, the robustness and reproducibility of this fluorescent homogeneous method were evaluated to determine whether this sensor would be convenient used for high-throughput screening assay. The Z' factor is an available criteria to quantify the suitability of a particular assay for use in a full-scale, high-throughput screening.[47-49] As shown in Figure 3C and 3D, the Z' factors of the assay for quercetin and curcumin were 0.80 and 0.86, respectively. These results indicate that the proposed method is suitable for SrtA inhibitor screening and has a clear potential in anti-virulence drugs

screening.

Selectivity and SrtA Detection in Human Serum.

Considering that SrtA of the gram-positive pathogenic bacteria plays a vital role in some human tissues infection, herein some important enzymes in human body, such as glutathione S-transferases (GST), alkaline phosphatase (ALP), lysozyme, thrombin, protein kinase A (PKA), β-galactosidase and casein kinase II (CK II) were chosen as the control group to verify the specificity of the assay. As depicted in Figure 4A, it can be observed that only SrtA can induce significant fluorescence response, while all the control proteins cause negligible signal, suggesting the good selectivity of the method toward SrtA.

The excellent sensitivity and selectivity of the developed method suggest that it might be directly applied for SrtA analysis in complex samples. SrtA under different concentrations (10, 50 and 100 nM) were spiked in human serum, and its activity was detected by the proposed assay. The analytical result shown in Figure 4B reveals that the results obtained by our method are almost coincident with the amount of SrtA we added, and the relative standard deviations (RSDs) of peak intensity were 0.48%, 0.96% and 0.74% in three repetitive assays of 10, 50 and 100 nM of SrtA, respectively. Hence the proposed method can be successfully applied to detect SrtA in complicated biological samples with good selectivity, accuracy and reproducibility.

Application in Bacteria Detection.

In gram-positive bacteria, the SrtA transpeptidases are anchored on the extracellular

side of the cell wall.[50,51] Hence, the potential applicability of the proposed SrtA assay in bacteria detection was evaluated further (Figure 5A). *S. aureus*, a typical gram-positive pathogen and a leading cause of hospital- and community-acquired infections that produce a wide spread of diseases, is chosen as a candidate.[52,53] As shown in Figure 5B (red column), significant fluorescence enhancement can be observed when *S. aureus* was mixed with the reaction buffer containing P_1, P_2 and GFP1-9 directly, while *S. aureus* only shows very weak fluorescence. Besides, when *S. aureus* was substituted by a gram-negative bacterium *E. coli* (blue column), which does not contain SrtA on the surface, no obvious fluorescence can be detected. On the basis of these results, the quantitative detection of *S. aureus* was carried out. As depicted in Figure 5C, the fluorescence intensity increases as the concentration of *S. aureus* rises from 1-10^7 CFU mL^{-1}, with a good linear correlation (R^2 = 0.993) in the concentration range of 10^2-10^5 CFU mL^{-1} (inset in Figure 5C). These results mean that our work could be applied for *S. aureus* bacteria detection.

The limitedly existing of *S. aureus* in frozen food was defined by the International Microbiological Specification Committee (ICMSF), which is not more than 10^4 CFU mL^{-1}.[54] Considering that the limit is in the linear range of our method for *S. aureus* detection, we try to measure *S. aureus* in quick frozen snacks (dumplings, glue pudding) and pasteurized milk by our method, with a standard method (Baird-Parker plate count method of live bacteria) as reference. No *S. aureus* was detected in these commercially available foods by both methods (data not shown), indicating that the foods are qualified. After being cultured, the number of bacteria increased (Figure 5D),

and the data detected by our method is comparable to that by the plate counting method. Moreover, less *S. aureus* was found in milk than that in the quick-frozen snacks, which is reasonable since milk is pasteurized (Figure 5D). The results indicate that our method can be successfully used to analyze whether there is an excess of *S. aureus* in real samples. Moreover, compared with the plate counting method that requires time-consuming germiculture (at least 24 h) and strict sterile operations, our one-pot solution method is simple and time-saving.

Conclusion

To summarize, we have developed a sortase-mediated tripartite split GFP assembly for SrtA activity detection and its inhibitor screening. The specific and effective linkage of P_1 and P_2 catalyzed by SrtA, and the high affinity between P_1-P_2 and GFP1-9, as well as low fluorescence background of GFP1-9, endow our assay excellent specificity and high sensitivity (LOD = 0.16 nM). Compared with traditional methods relied on fluorophore- and quencher-tagged peptide probes, HPLC or MS, our label-free and one-pot reaction method is simple and cost-effective. Moreover, the proposed approach has the great potential to be a high-throughput method for novel sortase-targeted anti-virulence drug screening, *in vitro* sortase activity analysis in human serum, and gram-positive pathogen detection. Furthermore, considering the flexible design and synthesis of the probe, this tripartite split GFP assembly-based method may be expanded to a versatile platform for the detection of different transpeptidase activities and their inhibitor screening.

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Notes

The authors declare no competing financial interest.

Supporting Information Available

Additional information, including the description of extensive method and figures, as noted in text.

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Figure Captions

Scheme 1. Schematic illustration of SrtA-catalyzed transpeptidation-mediated assembly of tripartite split GFP for label-free detection of SrtA activity.

Figure 1. (A) Fluorescence intensity of GFP1-9 under different conditions: GFP1-9 alone (column 1), with GFP10 and GFP11 (column 2), P_1 and P_2 (column 3), P_1-P_2 catalyzed by SrtA (column 4), and GFP10-11 (column 5). The concentrations of GFP1-9, GFP10, GFP11, P_1, P_2, SrtA and GFP10-11 are 2, 5, 40, 5, 40, 1 and 5 μM, respectively. (B) Characterization of the SrtA-catalyzed transpeptidation reaction by MALDI-TOF MS. The concentrations of P_1, P_2 and SrtA are 20 μM, 160 μM and 200 nM, respectively. (C) Native-PAGE analysis of GFP1-9, the assembly products of GFP1-9 with P_1-P_2 and GFP10-11, respectively, and GFP. The top is stained by coomassie brilliant blue, and the bottom is illuminated by UV lamp (365 nm).

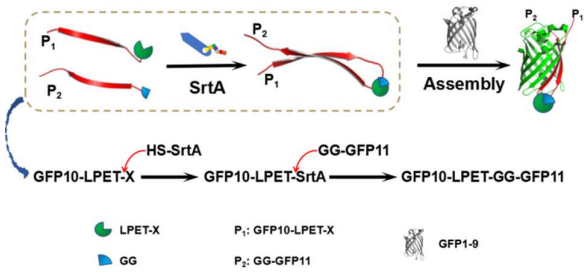
Figure 2. (A) Fluorescence spectra of the assembled GFP responds to SrtA under different concentrations (0-500 nM) in Tris buffer (50 mM Tris-HCl, 150 mM NaCl, 10 mM $CaCl_2$ and pH 7.5). Inset shows the fluorescence spectra under low concentrations of SrtA. (B) Calibration curve for SrtA detection. Inset shows the linear correlation of fluorescence response to SrtA concentration ranging from 0.3 to 100 nM. The concentrations of GFP1-9, P_1 and P_2 are 2, 5 and 40 μM, respectively.

Figure 3. Fluorescence spectra of the proposed assay in response to different concentrations of quercetin (0-300 μM) and curcumin (0-700 μM) (A and B). Inset shows the inhibition efficiency as a function of logarithmic concentration of quercetin

and curcumin. Evaluation of this approach for high-throughput screening of 50 μM quercetin (C) or 200 μM curcumin (D). The concentrations of GFP1-9, P_1, P_2 and SrtA are 2, 5, 40 and 0.05 μM, respectively.

Figure 4. (A) Fluorescence intensity responses of the sensor to different proteins. (1) SrtA; (2) GST; (3) ALP; (4) lysozyme; (5) thrombin; (6) PKA; (7) β-galactosidase; and (8) CKII. The concentrations of the proteins were all 50 nM. (B) Determination of SrtA in serum samples (n = 4). The concentrations of GFP1-9, P_1 and P_2 are 2, 5 and 40 μM, respectively.

Figure 5. (A) Schematic illustration of the proposed assay for label-free detection of gram-positive bacteria. (B) Fluorescence responses of the proposed assay to *S. aureus* and *E. coli* (10^8 CFU mL^{-1}). (C) Fluorescence intensity as a function of logarithmic concentration of *S. aureus*. (D) *S. aureus* number analysis in milk, glue pudding and dumplings using our method (red) and Baird-Parker plate count method (gray). The concentrations of GFP1-9, P_1 and P_2 are 2, 5 and 40 μM, respectively.


P1
P2
SrtA
P2
P1
Assembly
P2
P1
HS-SrtA
GG-GFP11
GFP10-LPET-X
GFP10-LPET-SrtA
GFP10-LPET-GG-GFP11
LPET-X
GG
P1: GFP10-LPET-X
P2: GG-GFP11
GFP1-9

Scheme 1

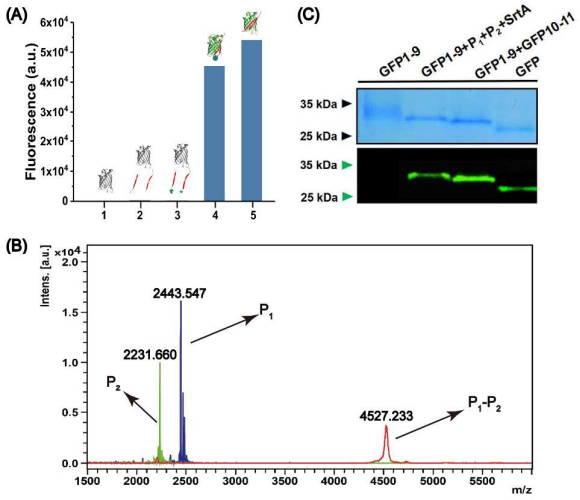

(A)
Fluorescence (a.u.)
6x10^4
5x10^4
4x10^4
3x10^4
2x10^4
1x10^4
0
1
2
3
4
5
(C)
GFP1-9
GFP1-9+P1+P2+SrtA
GFP1-9+GFP10-11
GFP
35 kDa
25 kDa
35 kDa
25 kDa
(B)
Intens. [a.u.]
x10^4
2.0
1.5
1.0
0.5
0.0
2443.547
P1
2231.660
P2
4527.233
P1-P2
1500
2000
2500
3000
3500
4000
4500
5000
5500
m/z

Figure 1

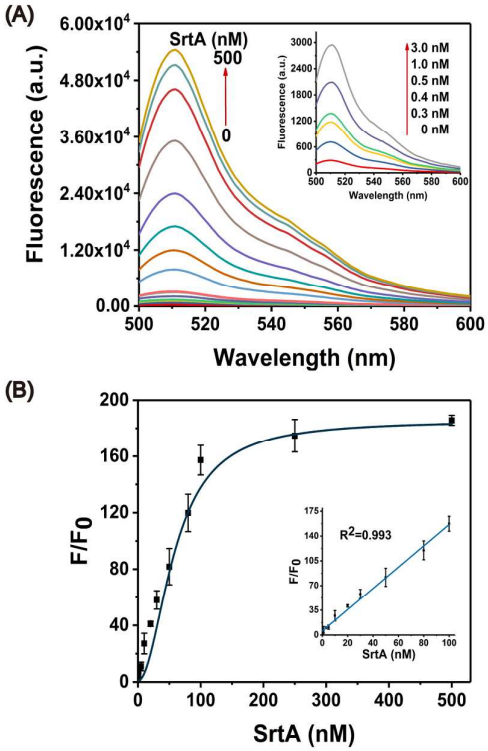

(A)
Fluorescence (a.u.)
6.00x10^4
4.80x10^4
3.60x10^4
2.40x10^4
1.20x10^4
0.00
SrtA (nM)
500
0
500 520 540 560 580 600
Wavelength (nm)
3.0 nM
1.0 nM
0.5 nM
0.4 nM
0.3 nM
0 nM
Fluorescence (a.u.)
Wavelength (nm)
(B)
F/F0
200
160
120
80
40
0
0 100 200 300 400 500
SrtA (nM)
R2=0.993
F/F0
SrtA (nM)

Figure 2

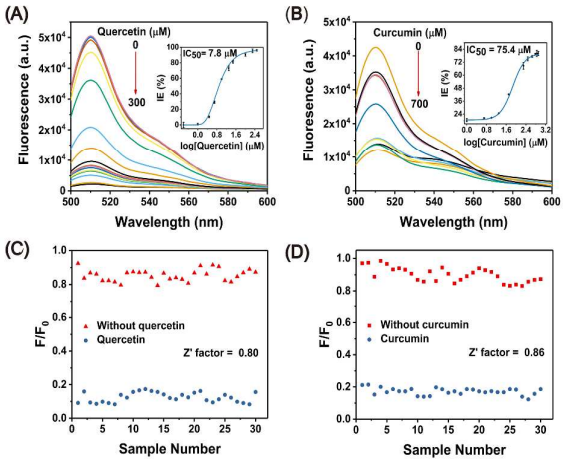

(A)
Quercetin (μM)
0
300
Fluorescence (a.u.)
Wavelength (nm)
IC50= 7.8 μM
IE (%)
log[Quercetin] (μM)
(B)
Curcumin (μM)
0
700
Fluorescence (a.u.)
Wavelength (nm)
IC50 = 75.4 μM
IE (%)
log[Curcumin] (μM)
(C)
F/F0
Without quercetin
Quercetin
Z' factor = 0.80
Sample Number
(D)
F/F0
Without curcumin
Curcumin
Z' factor = 0.86
Sample Number

Figure 3

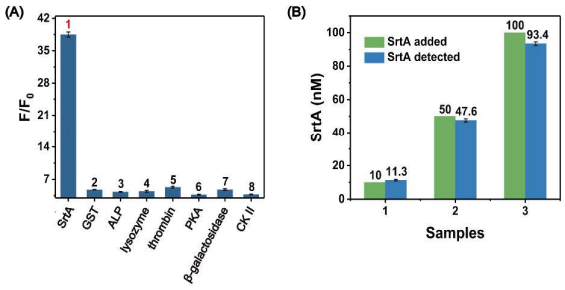

(A)
F/F0
SrtA
GST
ALP
lysozyme
thrombin
PKA
β-galactosidase
CK II
(B)
SrtA added
SrtA detected
SrtA (nM)
Samples
10
11.3
50
47.6
100
93.4

Figure 4

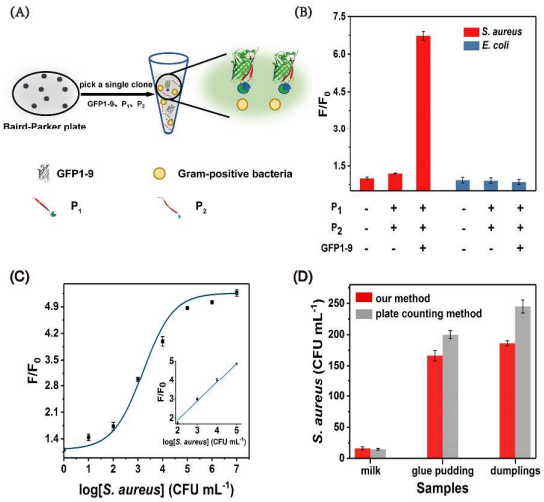
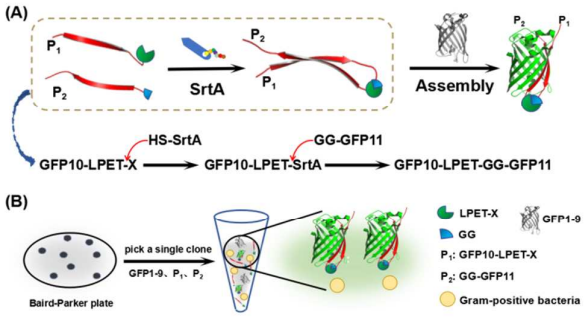

(A)
pick a single clone
GFP1-9、P1、P2
Baird-Parker plate
GFP1-9
Gram-positive bacteria
P1
P2
(B)
S. aureus
E. coli
F/F0
P1
P2
GFP1-9
(C)
F/F0
log[S. aureus] (CFU mL-1)
(D)
our method
plate counting method
S. aureus (CFU mL-1)
milk
glue pudding
dumplings
Samples

Figure 5

For TOC only


(A)
P1
P2
SrtA
P2
P1
Assembly
P2
P1
HS-SrtA
GG-GFP11
GFP10-LPET-X
GFP10-LPET-SrtA
GFP10-LPET-GG-GFP11
(B)
pick a single clone
GFP1-9、P1、P2
Baird-Parker plate
LPET-X
GG
GFP1-9
P1: GFP10-LPET-X
P2: GG-GFP11
Gram-positive bacteria