Supporting Information for:

Design of highly active substrates using molecular docking for microbial transglutaminase detection

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Additional Experimental Section

Additional Tables: Table S1

Additional Figures: S1-S9

Purification of mTGase : Superdex^{TM} 75 pg column (GE healthcare life sciences, UK) was washed with at least 5 column volumes of dH_2O and equilibrated with the same amount of 50 mM MES (pH 6.0) buffer. 30mg mTGase powder (donated by Jiangsu Yiming Biological Products Co., Ltd.) was dissolved in 30ml MES solution and filtered by 0.22 μM microporous membrane before loaded into the column. The column was washed with 50 mM MES (pH 6.0) buffer using AKTA purifier (GE Healthcare, UK) and mTGase was collected in 2 mL Eppendorf tubes. The purified mTGase was analysed using Polyacrylamide gel (SDS-PAGE) analysis to determine purity as shown in Figure S1. The concentration of mTGase used in the experiment was determined with a BCA protein Assay kit.

Table S1 | Amino acids appearing at each position around the central residue Gln (Q)

Position	-2	-1	0	+1	+2	+3
Types of amino acids	G,A,L,I,V,P F,M,W	P,W,F,Y,L,G R,K,A,V,I,M	Gln (Q)	A,S,R	P,R,A	Y

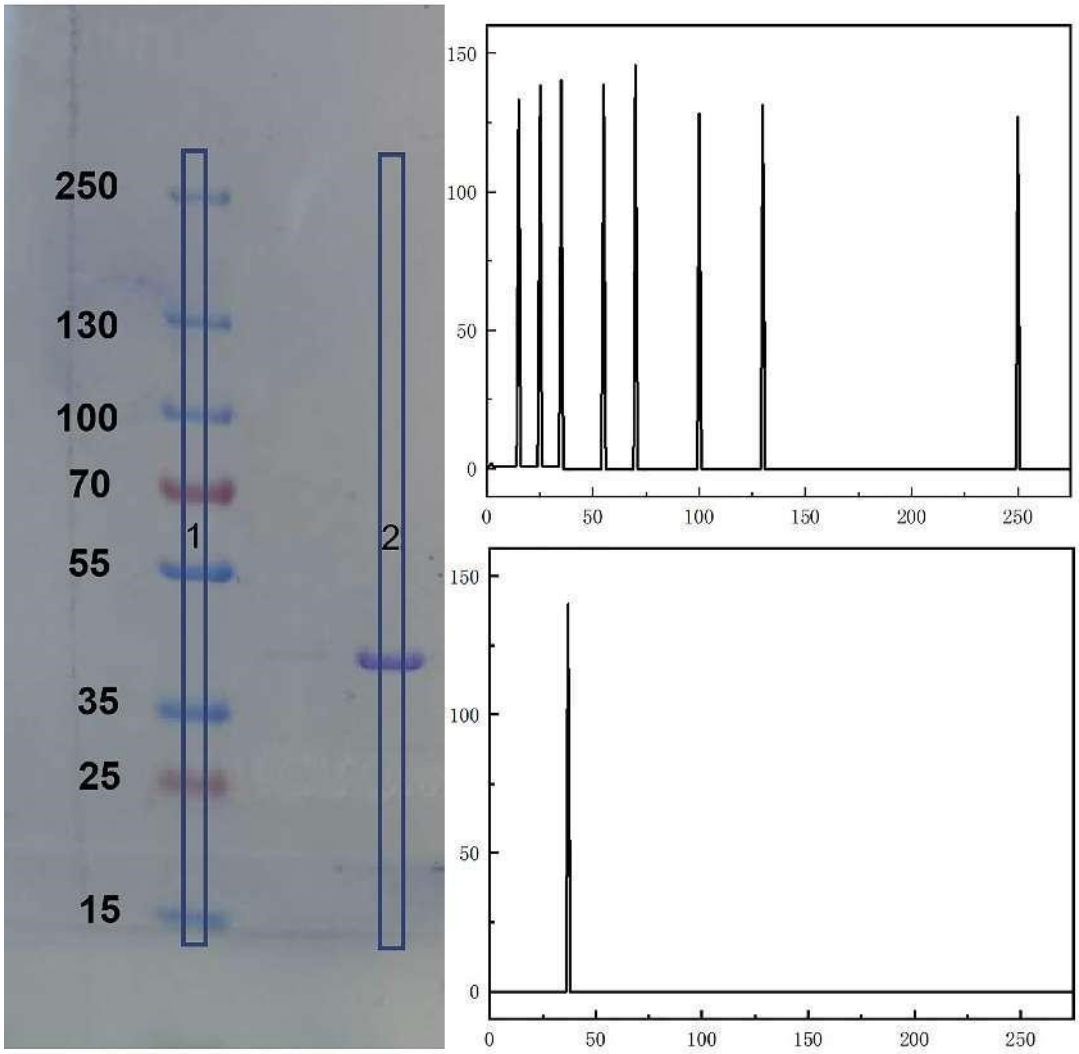

Fig. S1 Polyacrylamide gel (SDS-PAGE) analysis of mTGase. SDS-PAGE of purified mTGase (blue box 2) with a PageRuler Plus Prestained ladder (box 1) as reference. Image analysis using Fiji software calculated mass to be approximately 38 kDa.

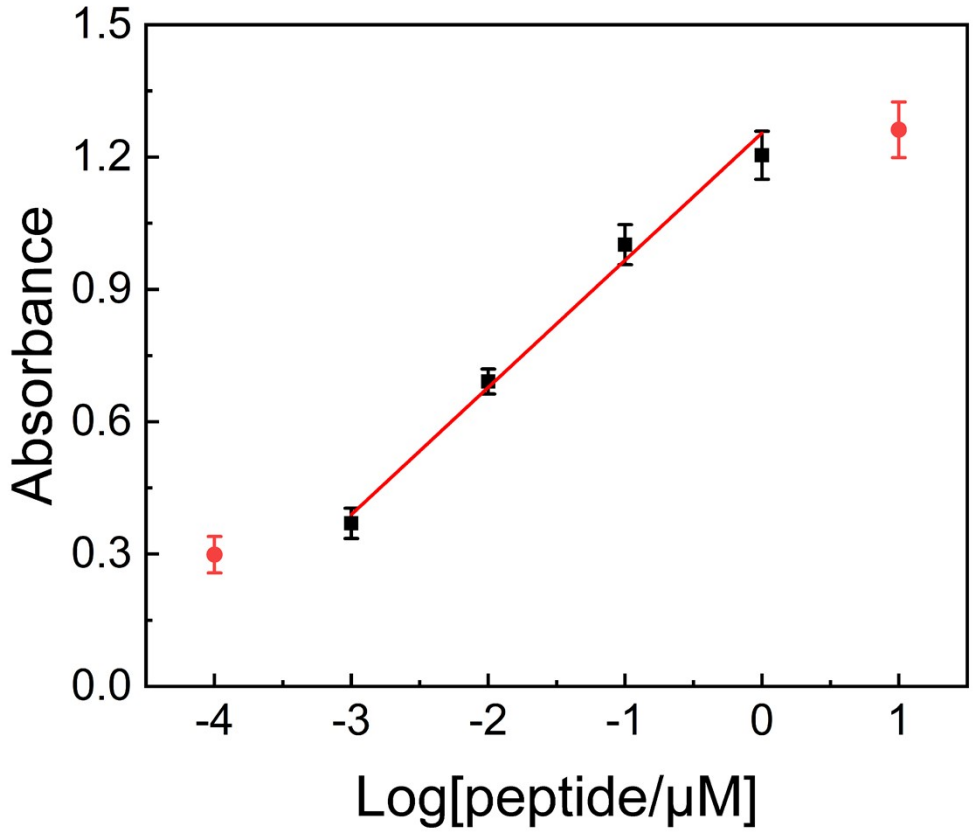

Fig. S2 Logarithmic plots of the integrated OD_{600} values as a function of the concentration of PA4+PD10 in MES buffer (50 mM, pH 6). The concentrations of peptides were from 0.0001 to 10 μM, the concentration of mTGase was 26 μM. Average OD_{600} value of CP has been subtracted from signal value (as shown in Fig. 1).

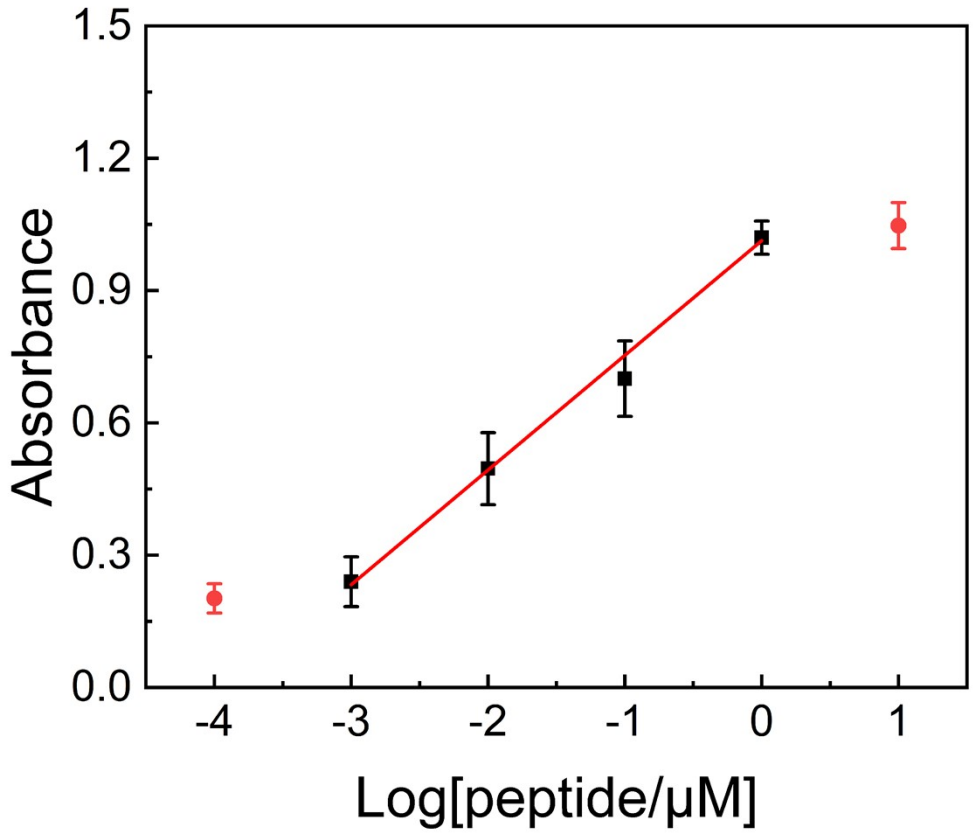

Fig. S3 Logarithmic plots of the integrated OD_{600} values as a function of the concentration of PA4+PD15 in MES buffer (50 mM, pH 6). The concentrations of peptides were from 0.0001 to 10 μM, the concentration of mTGase was 26 μM. Average OD_{600} value of CP has been subtracted from signal value (as shown in Fig. 1).

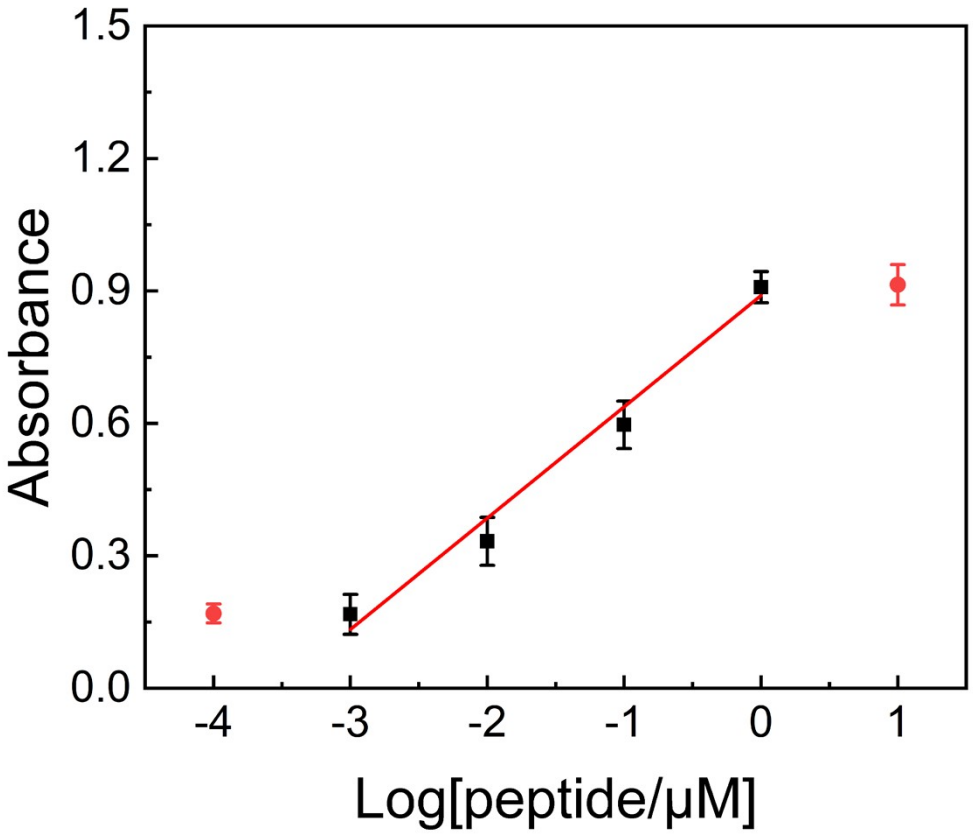

Fig. S4 Logarithmic plots of the integrated OD_{600} values as a function of the concentration of PA3+PD14 in MES buffer (50 mM, pH 6). The concentrations of peptides were from 0.0001 to 10 μM, the concentration of mTGase was 26 μM. Average OD_{600} value of CP has been subtracted from signal value (as shown in Fig. 1).

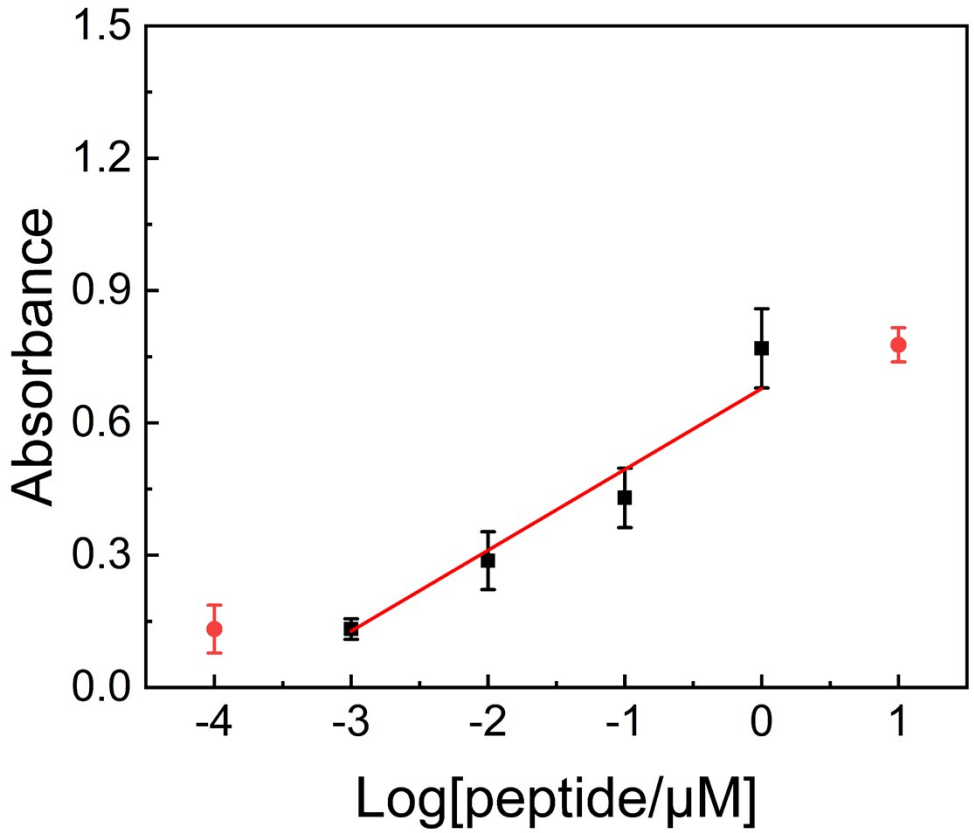

Fig. S5 Logarithmic plots of the integrated OD_{600} values as a function of the concentration of PA4+PD14 in MES buffer (50 mM, pH 6). The concentrations of peptides were from 0.0001 to 10 μM, the concentration of mTGase was 26 μM. Average OD_{600} value of CP has been subtracted from signal value (as shown in Fig. 1).

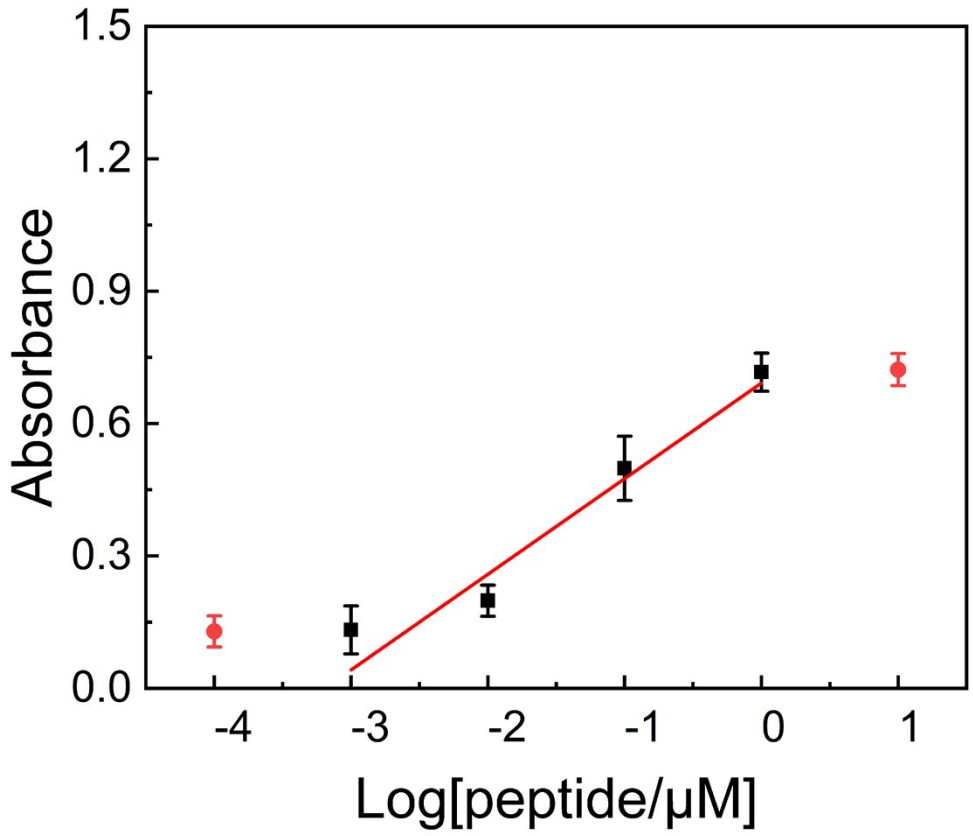

Fig. S6 Logarithmic plots of the integrated OD_{600} values as a function of the concentration of PA5+PD12 in MES buffer (50 mM, pH 6). The concentrations of peptides were from 0.0001 to 10 μM, the concentration of mTGase was 26 μM. Average OD_{600} value of CP has been subtracted from signal value (as shown in Fig. 1).

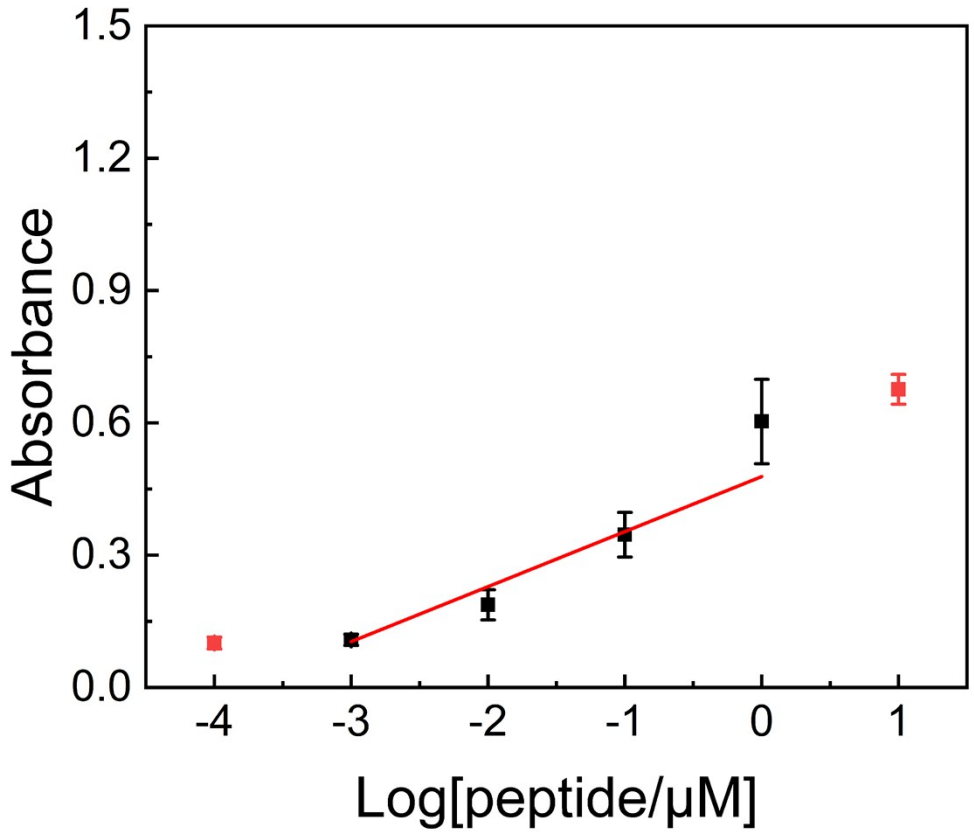

Fig. S7 Logarithmic plots of the integrated OD_{600} values as a function of the concentration of PA2+PD14 in MES buffer (50 mM, pH 6). The concentrations of peptides were from 0.0001 to 10 μM, the concentration of mTGase was 26 μM. Average OD_{600} value of CP has been subtracted from signal value (as shown in Fig. 1).

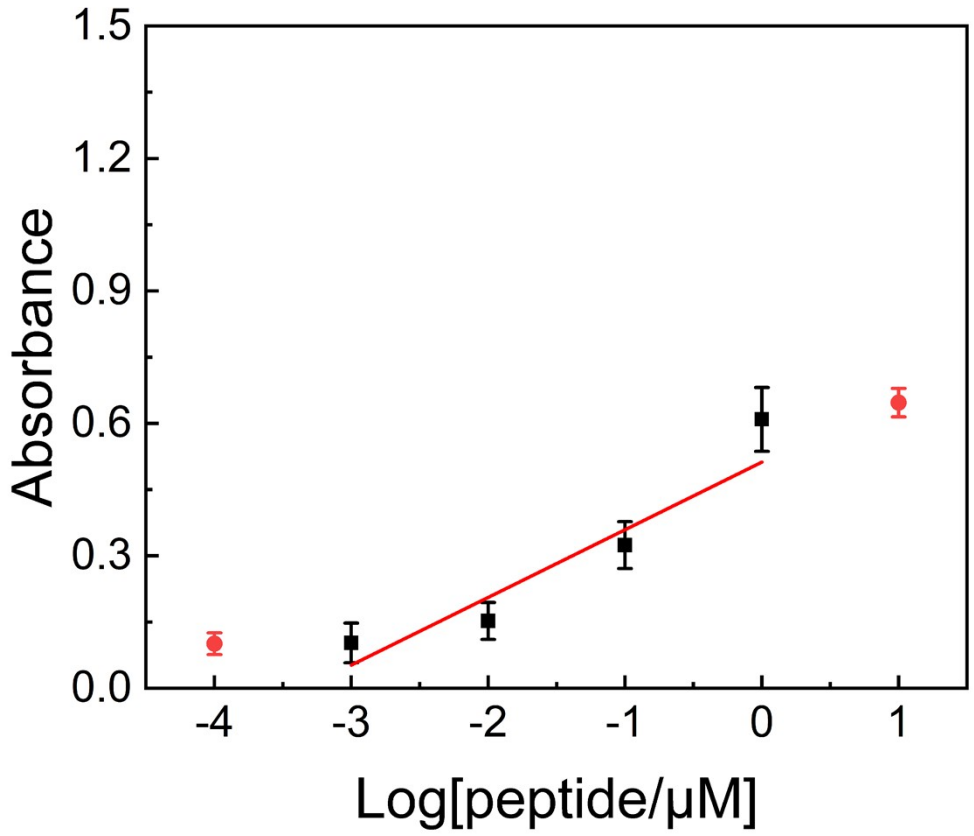

Fig. S8 Logarithmic plots of the integrated OD_{600} values as a function of the concentration of PA1+PD14 in MES buffer (50 mM, pH 6). The concentrations of peptides were from 0.0001 to 10 μM, the concentration of mTGase was 26 μM. Average OD_{600} value of CP has been subtracted from signal value (as shown in Fig. 1).

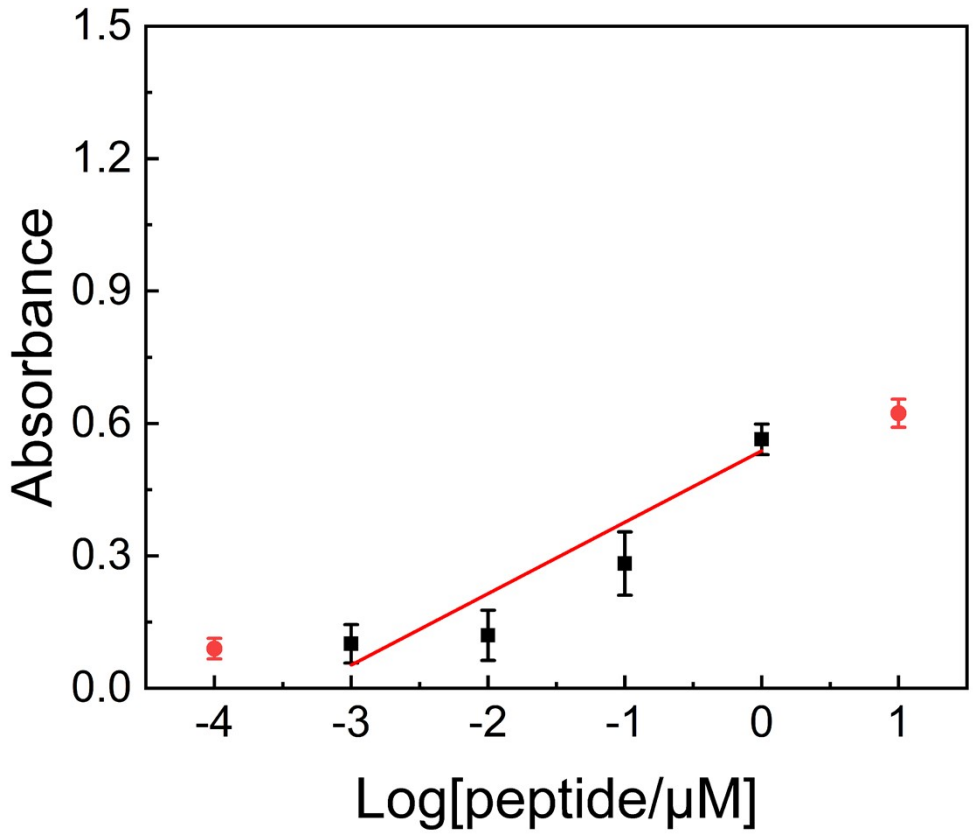

Fig. S9 Logarithmic plots of the integrated OD_{600} values as a function of the concentration of PA3+PD15 in MES buffer (50 mM, pH 6). The concentrations of peptides were from 0.0001 to 10 μM, the concentration of mTGase was 26 μM. Average OD_{600} value of CP has been subtracted from signal value (as shown in Fig. 1).