

## **Supplementary Information**

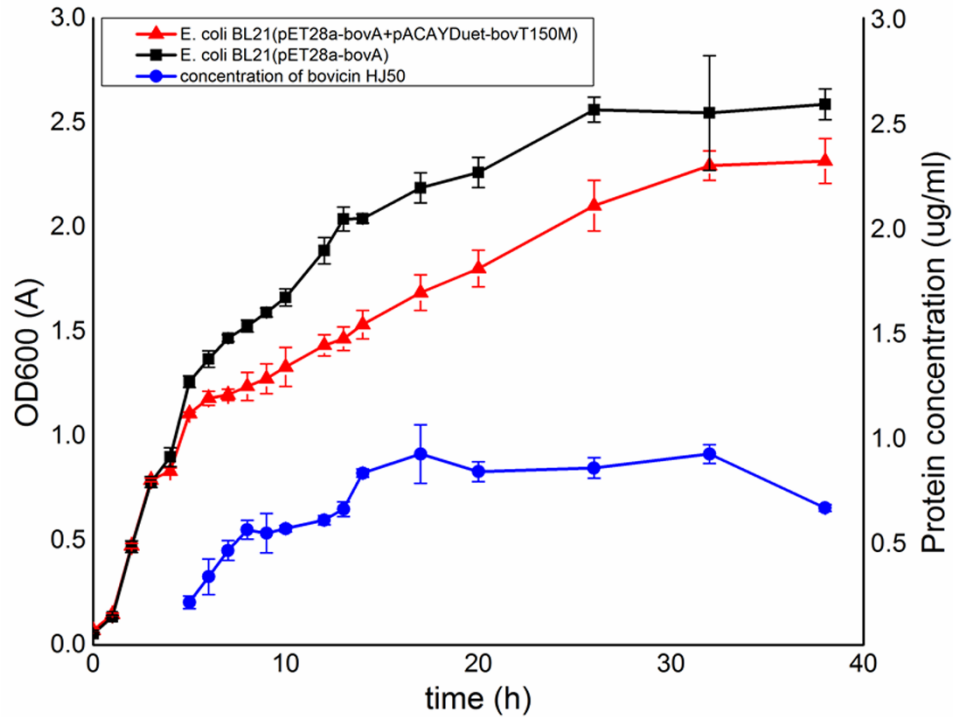
### **One-pot synthesis of class II lanthipeptide bovicin HJ50 via an engineered lanthipeptide synthetase**

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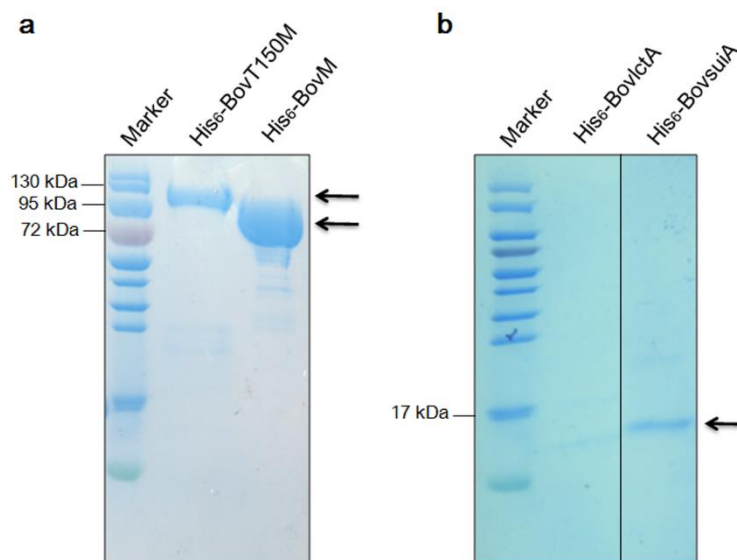
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**Figure S1.** Growth curve of *E. coli* cells and bovicin HJ50 production in the cell lysates. Black line, growth curve of *E. coli* BL21 (DE3) containing plasmid pET28a-bovA; red line, growth curve of *E. coli* BL21 (DE3) containing plasmids pET28a-bovA and pACYC-Duet-bovT<sub>150M</sub>; blue line, concentration of bovicin HJ50 in the cell lysates of *E. coli* BL21 (DE3) containing plasmids pET28a-bovA and pACYC-Duet-bovT<sub>150M</sub>. IPTG was added at 3 h and triplicates were recorded.



**Figure S2.** Identification of purified proteins by SDS-PAGE. (a) Identification of His<sub>6</sub>-tagged BovT150M (118.8 kDa) and BovM (101.2 kDa). (b) Purification and identification of His<sub>6</sub>-tagged BovlctA and BovsuiA.

**Amino acid sequence of recombinant protein BovT150M.** The His<sub>6</sub> tag was colored in gray, the BovT150 domain was colored in yellow and the BovM domain was colored in green. The crucial Cys15 of the enzymatic center of BovT150 domain and the conserved Asp264 of BovM domain are shown in red.

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMRKIKKEEIQFQQND**CGP**  
CCLSMILNYYDYQITVPELSSQLSKKNGWSMLDLKTVA AKYYLQSNVFKITD  
LKGFDKLQCPLVLFWESSHFVVLESISENIVIVDPSIGHVKLNRNEFNQKFSGF  
AMSFVKNEHFQQRKLSKLRVYVKEFNKA**GT**MEKLDLTINNESESFHKIIDNIVS  
DFYLVILDVAVQTNAELSNVHKYLYKNIRQVLLPILVQDINEWRLESKHQKNDT  
NQEYIDYCYLFISKNRFAYLKNKYELLNLRIDTISETKLNLNFLKNIDKSVSS  
LKKVFPQCDFEIKLKFIDFIGDNHGLYQSIMFEVSGKVFFYKCHGSEITNFIVT  
LQKEIPSLNFLKLPMTYIDQEFIIQEKVTHSSVLKDDIEEYHNMGKLLGILYL  
LNGNDMHNENIARGKNPIV**D**VETLINPIECSDSKMDDSIFSVGMLPMKYN  
RNFEGIFDTSSLGQSNIVSEKVFKEYKPFTSEIQLVLIDHTFSDLDRYLPRYKEIH  
FDVIDYLGVVEDGFIESYHLIMNHKKEIAKVISREASNINCRIVLRNTRIYSEIL  
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GVILVRYKFCQKLPENISELEQLMRQFMRLYKNSELKNGFAHGISGIKIIFYI  
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KENILNHLPRMLTYLIDLSDYCLCHGSFGVLDLFLTLTSQKELLPKEFHDEYVK  
LEKYHLEMITQCRILKKDVSLEFTGISGILYLN RKESGKNSVLTFGF.