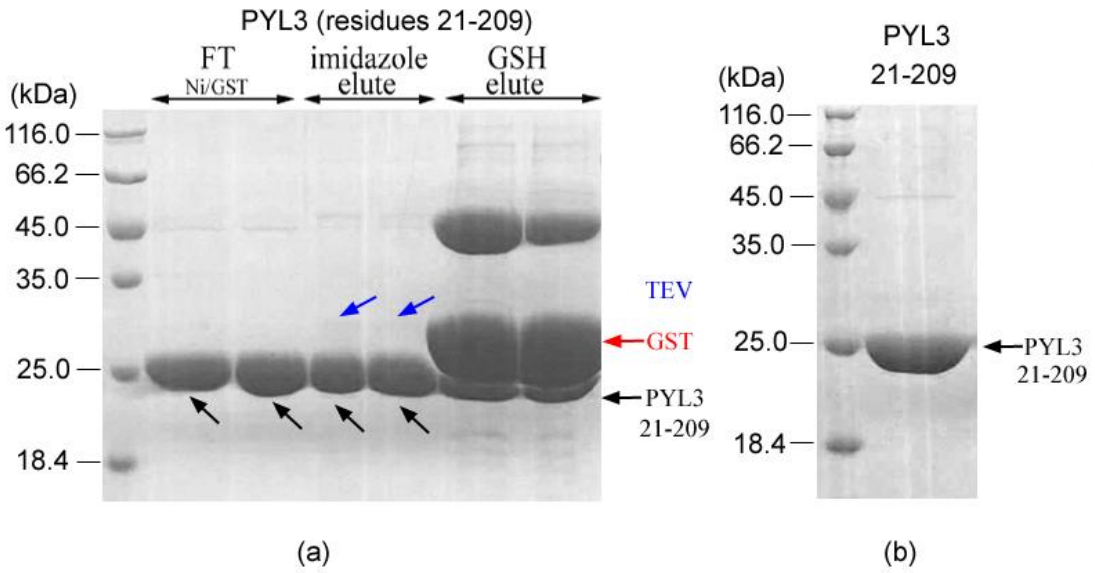
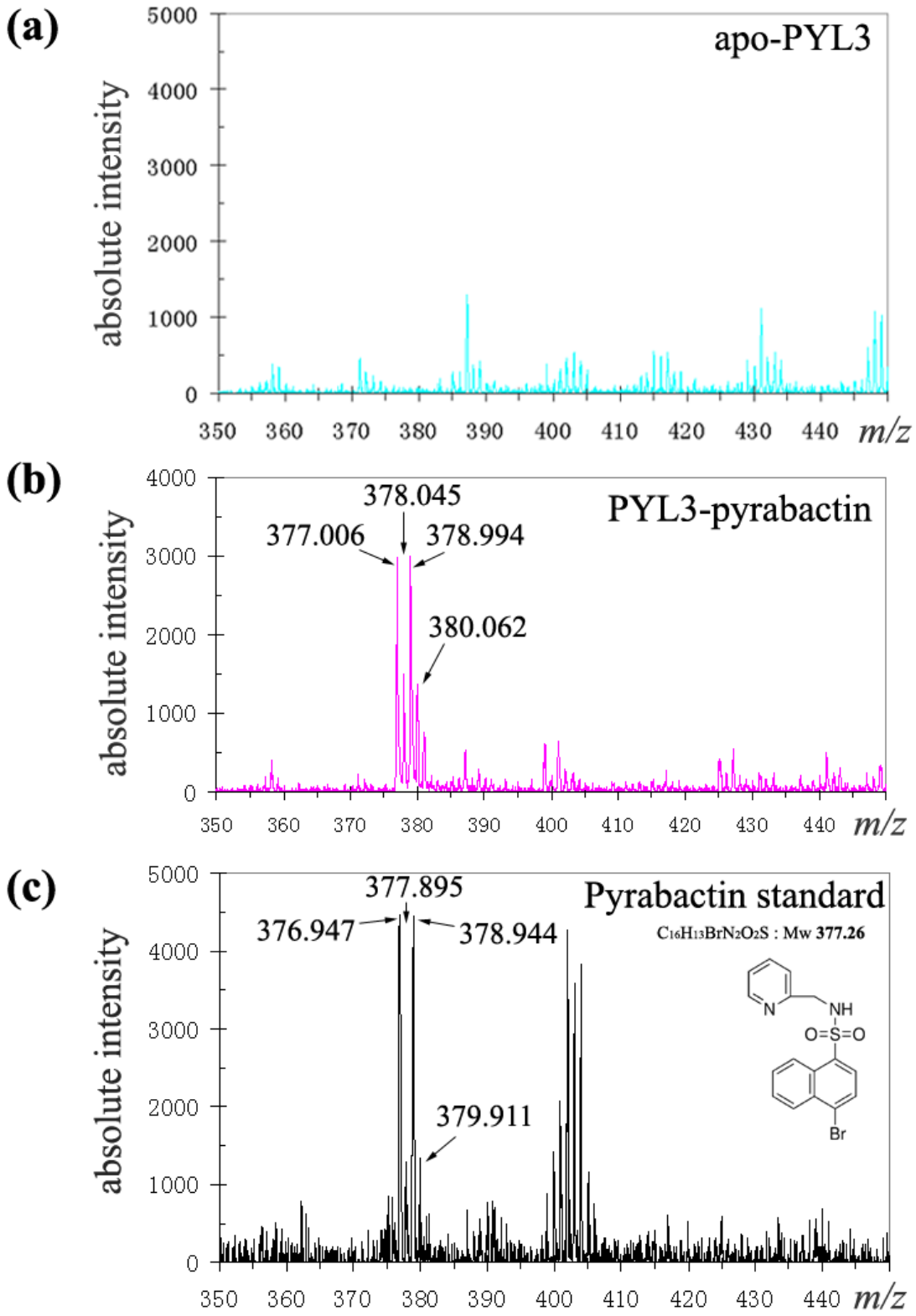
Supplementary Figure 1. PYL3 in different purification steps were analyzed by SDS-PAGE. (a) After GST fusion protein (residues 21-209) bound to a glutathione sepharose resin column, 0.5 mg His-Tag TEV protease was added into the column to excise GST-tag overnight. The reaction mixture flowed though Ni column and GST column (**FT**). Then, the Ni column and GST column were eluted by 500 mM **imidazole** and 10 mM reduced **GSH**, respectively. The FT and two eluates were subjected to SDS-PAGE. After cut by the TEV protease, PYL3 still partly bound both Ni column and GST column. The PYL3 (residues 21-209), TEV protease and GST tag were indicated by black arrow, blue arrow and red arrow, respectively. We only recovered FT for further purification. **(b)** A further purification step for FT was performed by size exclusion chromatography (Superdex 200, GE Healthcare).



Supplementary Figure 2. Mass spectrum analysis of the PYL3-pyrabactin complex crystals showed the existence of pyrabactin. **(a)** Apo-PYL3, **(b)** PYL3-pyrabactin crystal, **(c)** Pyrabactin standard. Apo-PYL3 in the buffer (150 mM NaCl, 20 mM Tris-HCl, pH7.5) was sufficiently dialyzed with 50 mM NH_4HCO3 buffer overnight. The concentration of the desalted apo-PYL3 was 5 mg/ml and 1ul aliquot of apo-PYL3 was spotted on the chip. Several crystals of the PYL3-pyrabactin complex were washed by the corresponding well buffer five times and then transferred into 2 µl aliquot of 50 mM NH_4HCO3 solution, which was spotted on the chip. The powder of pyrabactin standard (≥98% (HPLC), Sigma) was dissolved by DMSO and 1 µl aliquot of 1 mM pyrabactin was spotted on the chip. 5 mg/ml Sinapic acid was chosen as matrix and 1 µl aliquot of Sinapic acid was added on the sample spots for sample/matrix co-crystallizing. 1 µl aliquot of 0.1% trifluoroacetic acid was used to soak the sample/matrix co-crystal one or two seconds and removed for desaltion. Detection by MALDI-TOF mass spectrometry (autoflex II TOF/TOF, Bruker Daltonics) was carried out. The several peaks near *m/z*=377 were the classic isotope peaks for Br atom in pyrabactin. Obviously, the mass spectrum results demonstrated the existence of pyrabactin in the PYL3-pyrabactin complex crystal.


(a)
apo-PYL3
absolute intensity
5000
4000
3000
2000
1000
0
350 360 370 380 390 400 410 420 430 440 m/z
(b)
PYL3-pyrabactin
378.045
377.006
378.994
380.062
absolute intensity
4000
3000
2000
1000
0
350 360 370 380 390 400 410 420 430 440 m/z
(c)
Pyrabactin standard
377.895
376.947
378.944
379.911
C16H13BrN2O2S : Mw 377.26
NH
O=S=O
Br
absolute intensity
5000
4000
3000
2000
1000
0
350 360 370 380 390 400 410 420 430 440 m/z