Supporting Information --- Supplementary information, Data S1

SI Materials and Methods

Protein Expression and Purification.

The extracellular LRR domain of Arabidopsis PEPR1 (residues 1-767, PEPR1LRR) with a C-terminal 6×His tag was cloned into the pFastBac™ 1 vector (Invitrogen). The protein was expressed using the Bac-to-Bac baculovirus expression system (Invitrogen) in high five cells. One litre of cells (1.8×10^6 cells ml^{-1} cultured in the medium from Expression Systems) was infected with 20 ml baculovirus. 48h after the infection, the media was harvested for purification of the secreted proteins using Ni-NTA column (Novagen) followed by gel filtration chromatography (Hiload 200, GE Healthcare). Methods previously described were used to express and purify the extracellular LRR domain of BAK1 from *Arabidopsis* (residues 1-220, BAK1LRR). For crystallization, the purified AtPEPR1LRR protein was concentrated to about 7.0 mg ml^{-1}.

Crystallization, Data collection, Structure Determination and Refinement.

Crystallization experiments were performed with hanging-drop vapor-diffusion methods by mixing equal volumes of protein and reservoir solution at 18℃. A mixture of PEPR1LRR (~7.0mg ml^{-1}) and the chemically synthesized AtPep1 peptide (residues 1-23, China Scilight Biotechnology, 20 mg ml^{-1}) with a molar ratio of 1: 5 was used for crystallization. Initial crystals of AtPEPR1-ECD were obtained in buffer containing 0.2 M Trimethylamine N-oxide dehydrate, 0.1 M Tris pH 8.5, 20% w/v Polyethylene Glycol Monomethyl ether 2000. However, the crystals diffracted X-ray poorly. To improve the diffraction ability of the crystals, the PEPR1LRR protein was digested with endoglycosidase F1 and F3 at 18℃ overnight and further cleaned using gel filtration. Similar conditions produced crystals of the de-glycosylated protein, which well diffracted X-ray. Diffraction data were collected on the BL17U1 beam-line of the Shanghai Synchrotron Research Facility (SSRF). The datasets were processed using HKL2000 (1). The structure was determined by the molecular replacement method with PHASER (2) and refined with PHENIX (3). The structure of FLS2LRR (PDB code: 4MN8) was used as the initial searching model. After refinement of the initial model, AtPep1 (residues 7-23) was built into the electron density in COOT (4). The structure of PEPR1LRR-AtPep1 was finally refined to a resolution of 2.59 Å with R_{factor}23.7% and R_{free} 28.8%, respectively. All the figures representing structures were prepared with PyMOL.

Gel Filtration Assay.

PEPR1LRR and BAK1LRR proteins purified as described earlier were subjected to gel filtration analysis (Hiload 200, GE Healthcare) in the absence or presence of the chemically synthesized AtPep1 peptide (from *Arabidopsis*). Approximately equal moles of AtPEPR1LRR, AtBAK1LRR and Pep1 peptide were incubated at 4℃ for 30 min before the gel filtration analysis. Buffer containing 10 mM Bis-Tris pH6.0, 100 mM NaCl was used for the assay. The assays were performed with a flow rate of 0.8 ml min^{-1} and an injection volume of 40 ml at 4°C. The proteins were visualized to SDS-PAGE and followed by Coomassie blue staining.

In vitro Pull-down Assay and Western blot.

The PEPR1LRR and BAK1LRR proteins were purified as described above. GST-AtPep1 and other AtPeps were expressed *E. Coli.* and purified using Glutathione Sepharose 4B beads (GS4B, GE Healthcare). The purified GST-tagged AtPep1 (from *Arabidopsis*) protein was used to pull down the His-tagged PEPR1LRR (residues 1-767) and the His tagged-BAK1LRR (residues 1-220) proteins. About 50 μg GST tagged-AtPep1 was first loaded onto 50 μl GS4B beads. After extensively washed with washing buffer (10 mM Bis-Tris pH6.0, 100 mM NaCl), the beads were incubated with the purified His-tagged PEPR1LRR or/and BAK1LRR proteins on ice for 20 min, followed by washing with 1 ml washing buffer for 3 times. The final beads were detected by Coomassie blue staining or western blot. Primary and secondary antibodies used for western blot were purchased from Beijing CoWin Bioscience Corporation (China). The assays were repeated for three times.

The GST tagged-AtPep5 protein was expressed in *E.coli* and purified as described above. The GST tag was then removed using prescission protease. The protein thus generated was further purified through gel filtration chromatography (Superdex 200, GE Healthcare). Approximately 50 μg of the His-tagged PEPR1LRR protein was added to mixtures containing an equal amount of GST tagged-AtPep1 protein and varying concentration of AtPep5 peptide. The mixtures were then individually loaded onto GS4B beads. After incubation on ice for 20 min, the beads were washed with 1 ml buffer containing 10 mM Bis-Tris pH 6.0, 100 mM NaCl for 3 times. The final beads were detected by Coomassie blue staining.

Structural Modeling of the PEPR1LRR-AtPep1-BAK1LRR Complex.

The complex formed by PEPR1LRR, BAK1LRR and AtPep1 was predicted by the docking method HoDock (5), which incorporates an initial rigid docking and a refined semi-flexible docking. The structure of the FLS2LRR-flg22-BAK1LRR complex (PDB code: 4MN8) was used as restraints for conformational searching and model selecting. Totally 11,000 complex structures were generated and scored to pick up the final correct complex structure model. Molecular dynamics simulation package Gromacs 4.52 (6) with OPLS force field was used for the minimization to relax and equilibrate the structure in solution. Then the minimized structure in the last frame fitting best with stereo-chemical restraints was selected as the built model.

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