Supplementary information, Data S1 Materials and Methods

Experimental Methods

Protein expression and purification

The LRR domain of TMK1 (residues 1–469; TMK1-LRR) from *Arabidopsis* with an engineered C-terminal 6×His tag was generated by standard PCR-based cloning strategy and its identity was confirmed by sequencing. The protein was expressed in sf21 cells using the vector pFastBac 1 (Invitrogen) with a modified N-terminal Hemolin peptide. One litre of cells (2.5×10^6 cells mL^{-1}) was infected with 20 ml baculovirus using a multiplicity of infection of 4 at 28 °C, and protein was harvested from the media after 48 h. The protein was purified using Ni-NTA (Novagen) and size-exclusion chromatography (Superdex 200, Pharmacia) in buffer (10 mM Tris, pH 8.0, 100 mM NaCl). Samples from relevant fractions were applied to SDS–PAGE and visualized by Coomassie blue staining. Protein purification was performed at 4 °C. For crystallization of TMK1-LRR, the purified protein was concentrated to about 5.0 mg/mL in buffer containing 10 mM Tris, pH 8.0, 100 mM NaCl.

Crystallization

Crystals of TMK1-LRR were generated by mixing the protein with an equal amount of well solution (1.5 μL) by the hanging-drop vapor-diffusion method. Crystals of TMK1-LRR grew under the conditions of 0.15 M NaCl and 22% (w/v) polyethylene glycol (PEG) 3,350. Crystals grew to their maximum size ($0.1 \times 0.1 \times 0.1$ mm^3) within three weeks at 18 °C.

Data collection, structure determination and refinement

For data collection, crystals were equilibrated in a cryoprotectant buffer containing reservoir buffer plus 10% (v/v) glycerol. All the diffraction data sets were collected at the Shanghai Synchrotron Radiation Facility, beamline BL17U, and were integrated and scaled with HKL2000 [1]. Crystals of TMK1-LRR belong to space group P1 with one protein molecule per asymmetric unit. Molecular replacement (MR) with the program PHASER included in CCP4 [2] was used to solve the crystal structure of TMK1-LRR using the coordinates of BRI1 (residues 280-502) as the searching model. The missing residues were built into the density calculated using the refined model from MR with the program COOT [3]. The full model was subsequently subjected to further refinement by the program PHENIX [4]. The finally refined model contained 404 amino acids (residues 24-447). All the structure figures were prepared using PyMOL [5].

References

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