Genes encoding ankyrin domains of KANK1 (residues 1080–1329) were synthesized by Sangon Biotech (Shanghai) and cloned into a modified pET28-MHL (GenBankTM accession number EF456735). Expression plasmid was transformed into E. coli BL21 (DE3), and was overexpressed at 16 °C for 18 h in the presence of 1 mM isopropyl 1-thio- -D-galactopyranoside. ankyrin domain of KANK1 was first purified by a fast flow nickel-nitrilotriacetic acid column(GE Healthcare). N-terminal His6 tags of recombinant protein was removed by tobacco etch virus protease. Then the cleaved recombinant proteins were further purified by Superdex 75 gel filtration and mono Q ion exchange (GE Healthcare). The purified protein was concentrated to 15 mg/mol and stored at -80 °C.

The apo-form of the KANK1 ankyrin domain(residues 1080–1329) was crystallized by mixing an equal volume of 15 mg/ml protein with crystallization buffer (0.1M HEPES, pH 7.5, 0.2M lithium sulfate monohydrate, and 25% PEG 3350).Before flash-freezing crystals in liquid nitrogen, all crystals were soaked in a cryoprotectant consisting of 90% reservoir solution plus 10% glycerol.