ABSTRACT. Prion diseases are infectious, progressive, and fatal neurodegenerative disorders that occur in both humans and animals. The misfolded, denaturant resistant prion protein (PrPSc) propagates via self-templated conversion from the native fold (PrPc) into a β-sheet rich fold. Despite numerous models generated based on experimental evidence from a variety of structural biology techniques, an atomic structure of a mammalian prion fibril has yet to be realized. Here I present a cryo-EM structure of the protease resistant core of a recombinant human prion fibril composed of a pair of identical protofibrils. Helical reconstruction of prion fibril images reveals maps that inform the unambiguous identity and connectivity of residues 106-145 in the filament core. In each protofibril, the prion protein chain rises two layers (9.8Å) from one terminus to the other, creating intermolecular contacts that span three protein layers. This feature is thusfar unique to prion-like amyloid filament structures and may offer insight into the trademark stability of prion and prion-like fibrils.