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Crystal Structure of Hexameric PilT from *A. aeolicus*. Kenneth A. Satyshur, G.A. Worzalla, and K.T. Forest. Dept. of Bacteriology, Univ. of Wisconsin, Madison, WI.

PilT is a cytoplasmic hexameric ATPase associated with Type IV pilus retraction in Gram negative bacteria. PilT contains two domains, N and C terminal. ATP is bound in the C terminal domain's classic Walker A box as found in Rec A type hydrolases.

The membrane-associated N terminal domain is more closely associated with the C terminal domain of its neighbor than with its own C terminal domain. This domain packing arrangement imparts the hexamer with a minimum core opening of approximately 33 Angstroms within the C-terminus, and a maximum of 42 Angstroms at the membrane-associated N terminal domains. Invariant amino acids near the C-terminus, previously shown to be critical for function but not for ATPase activity, form a short alpha helix exposed on the cytoplasmic face of the C-terminal domain.

Crystals of the 250 amino acid C-terminal domain have been obtained in space group P41 and display nearly perfect two-fold twinning along the a axis. Synchrotron data were collected on SelenoMet derivatives to 1.87 Å resolution on BioCars 14-ID-B at the Advanced Photon Source. Selenium sites and initial phases were determined using Solve, and an initial chain trace was generated automatically using Resolve. Twin refinement within CNS and Shelx was used to refine the structure to the current R/Rfree of 18/25%. The details revealed by the C terminal domain structure aided in solving and refining the full-length PilT structure at 2.8 Å resolution.

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