

binding/modifying domains including a helix–turn–helix (HtH) domain that was not predicted from primary sequence. In addition, we have quantitatively examined the ability of Tex to bind various nucleic acid substrates and have found that Tex has a strong preference for single-stranded RNA (ssRNA). Binding appears to be sequence nonspecific, and mutagenesis studies indicate that this interaction is mediated by the flexible S1 domain. In contrast to an earlier proposal,^{1,2} we do not observe significant nuclease function associated with the Tex YqgF domain. Our findings provide a structural foundation for understanding Tex function and can guide future studies on the structure and function of Spt6.

Results and Discussion

Structure determination and overall description

The full-length *P. aeruginosa* Tex protein was expressed recombinantly in *Escherichia coli* and purified by Ni-chelate, heparin affinity, and gel filtration

chromatography. The C-terminal hexahistidine tag was retained for the structural and biochemical studies. The structure was determined by single-wavelength anomalous dispersion phasing and density modification using data collected to 2.7 Å resolution from a selenomethionine (Se-Met)-substituted crystal. This structure was refined against data collected from an isomorphous native crystal (crystal form I, 2.5 Å resolution) and from a second native crystal that belonged to the same space group but had substantially different cell dimensions (form II, 2.3 Å). The native structures were refined to R/R_{free} values of 24.0/27.4% (form I) and 22.1/26.6% (form II), with good geometry (Table 1). In both crystal forms, residues 1–730 are clearly observed in the electron density, with the exception of a short loop region (residues 246–251) in crystal form II. The 55 C-terminal residues of Tex and the hexahistidine tag are disordered and are not included in the final model.

Tex is ~53% α -helical and 10% β -sheet. The overall structure is notably flat and elongated, with approximate dimensions of $27 \times 72 \times 107$ Å (Fig. 1). The most striking structural feature is a long, central helix (H15) spanning ~72 Å and comprising amino acid

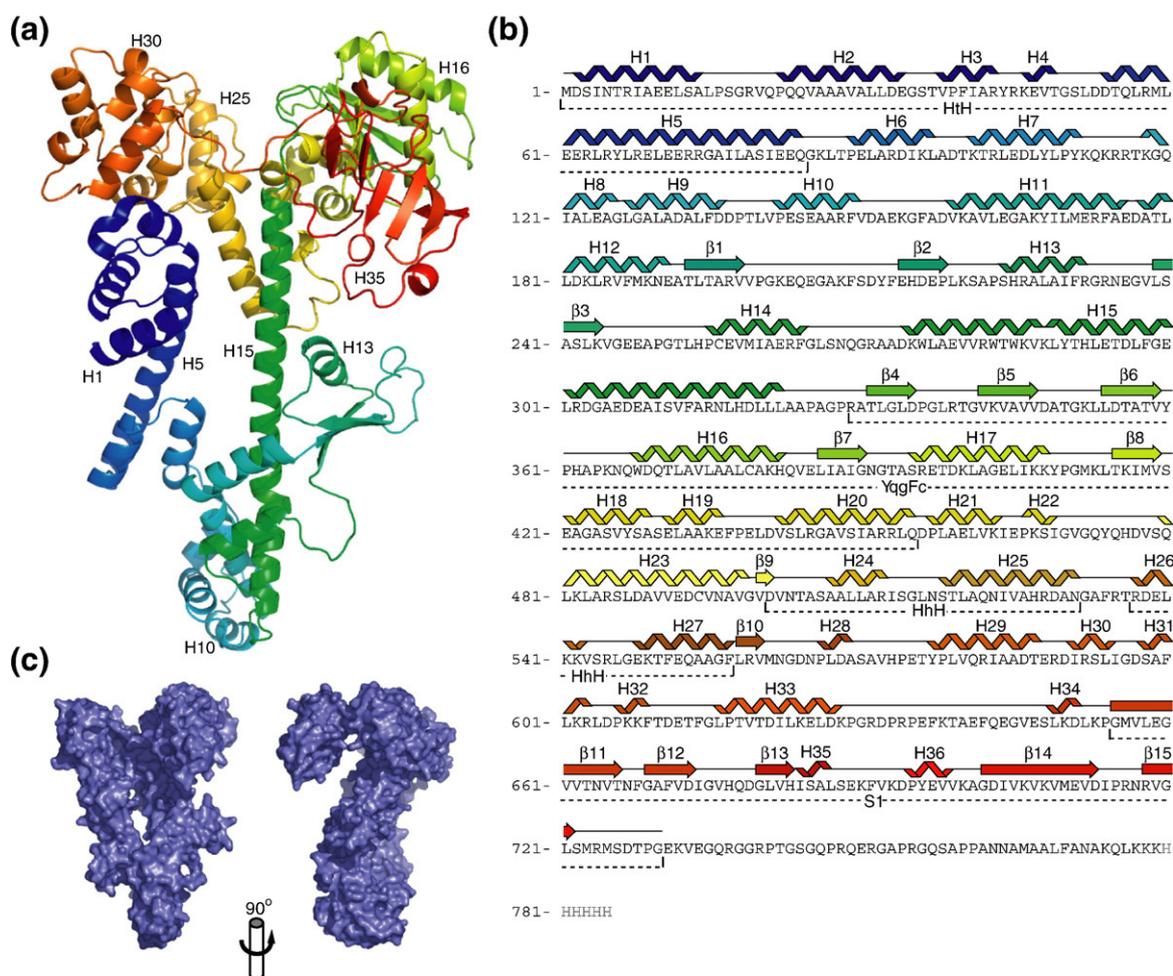


Fig. 1. Crystal structure of Tex. (a) Structure of Tex. The structure is colored from N-terminus (blue) to C-terminus (red). (b) Tex amino acid sequence. The observed secondary structure is indicated above the sequence. Identified domains are indicated below the sequence. (c) Tex surface representation in two orthogonal views. Left-hand view is the same as (a).