Supplementary Information

Structure of an Aprataxin–DNA complex with insights into AOA1 Neurodegenerative Disease

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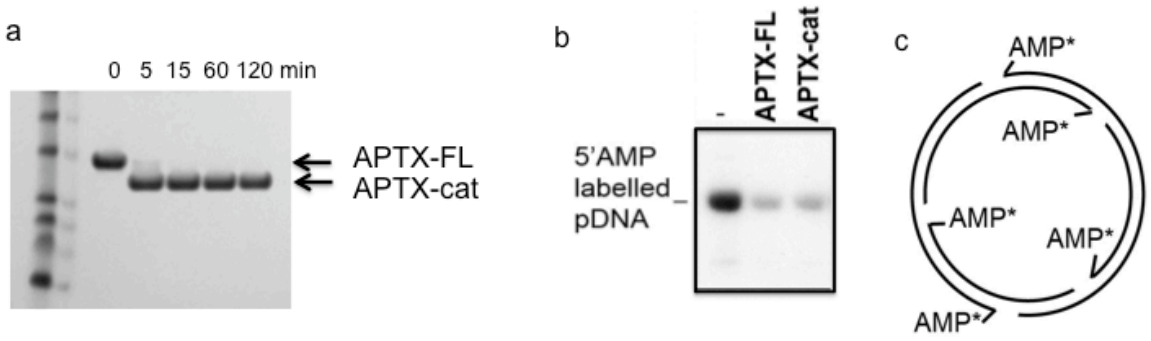
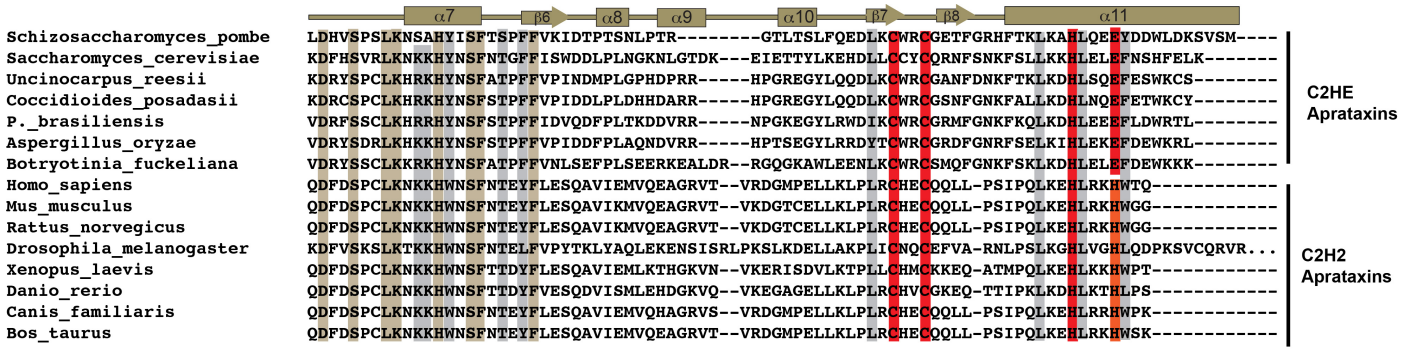
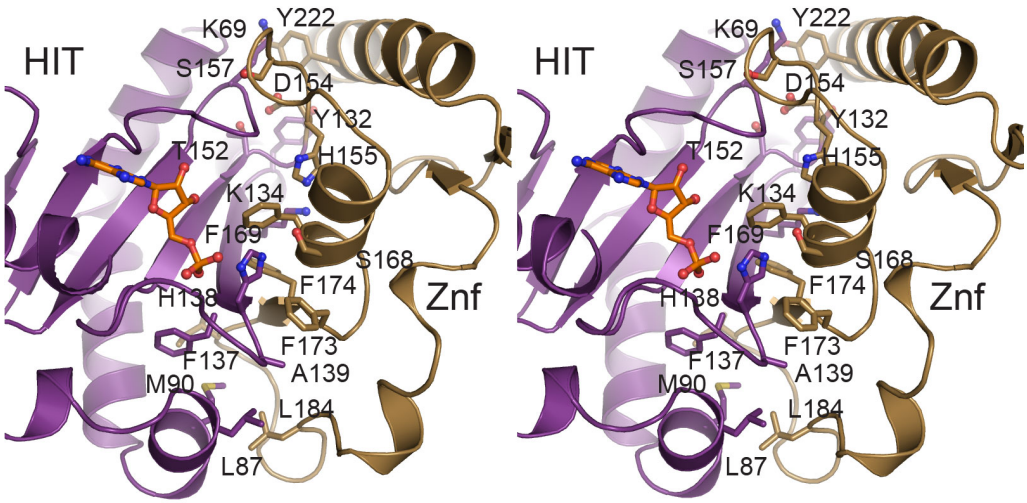


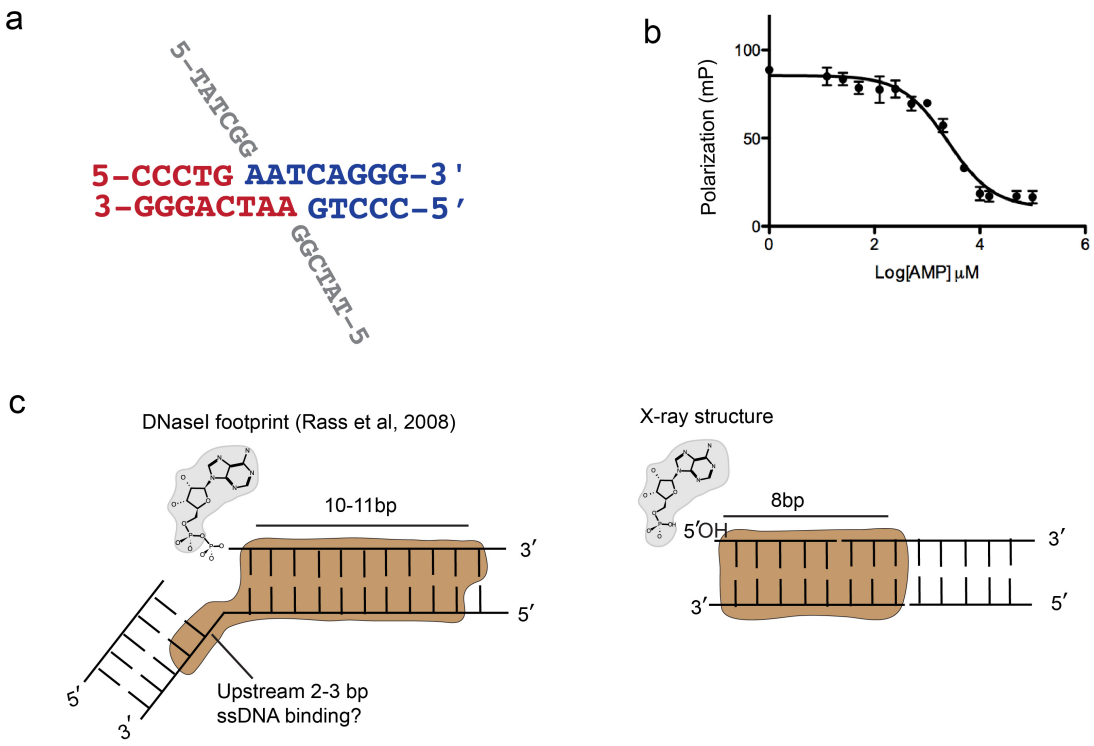
Supplementary Fig 1. Mapping structured and catalytically active DNA deadenylase domain in SpAptx. (**a**) Trypsin digestion time course of SpAptx. A trypsin cleavage site at Arg30 was identified with Maldi-TOF mass spectrometry. (**b**) Plasmid deadenylase activity of $SpAptx^{FL}$ and truncated catalytic domain ($Aptx^{cat}$). The truncated $Aptx^{cat}$ used in crystallization experiments displays similar activity to $Aptx^{FL}$. Plasmid deadenylation was monitored by measuring decrease in α-P^{32}-AMP incorporated into a nicked ΦX174 plasmid by abortive ligation. (**c**) Schematic of adenylated plasmid substrate used in DNA deadenylation reactions. Abortive ligation and deadenylation reactions performed as described in the methods.



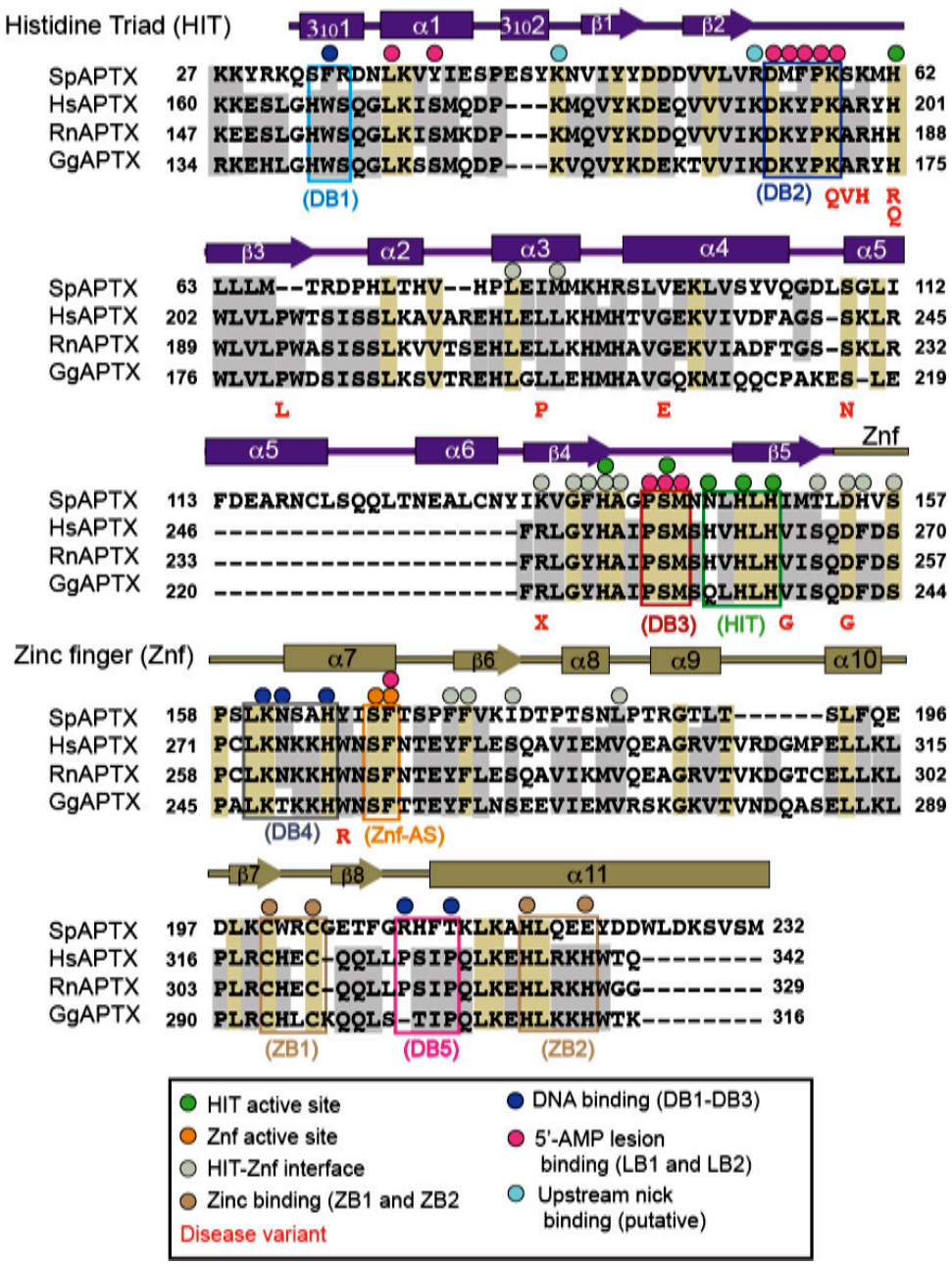
Supplementary Fig. 2. Structure based sequence alignment of the Aprataxin Znf DNA binding region. Conserved Zn binding residues are highlighted in red. Fungal aprataxins bear a C_2HE Zn binding core, whereas vertebrate APTX homolgs are C_2H_2 (orange highlight).



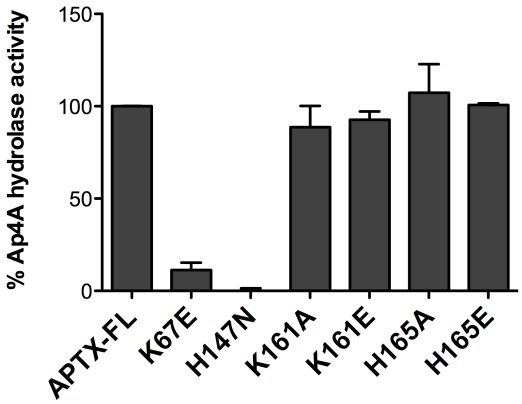
Supplementary Fig. 3. Structure of the HIT-Znf interface. Residues conserved at the HIT-Znf interface are shown. HIT domain is in purple, Znf in gold. Bound AMP is shown in orange. DNA is omitted for clarity. A stereo view is displayed.



Supplementary Fig. 4. DNA substrates and bound DNA for crystallization of the Aptx–DNA–AMP–Zn complex. (**a**) DNA sequence of crystallized oligonucleotides. Blue and red regions reflect ordered bases, whereas grey bases were not observed in the crystals. (**b**) AMP is a competitive inhibitor of Aptx binding to a 5' phosphorylated SSB substrate. IC50 (AMP)=2.4mM. APTX wt full-length protein (200nM) was mixed with AMP at concentrations ranging from 0 – 100mM, and competition for DNA binding was monitored by measuring the decrease in fluorescence polarization (see methods). IC50 (AMP)=2.4mM was calculated by fitting to a one site competition model in Graph Pad Prism. (**c**) Comparison of DNAseI footprint of hAptx on a gapped adenylate, and the DNA binding footprint defined by the Aptx–DNA–AMP–Zn complex structure.



Supplementary Fig. 5 Aptx structure-based sequence alignment. Secondary structure for the HIT domain (purple) and Znf domain (brown) is displayed above ClustalW sequence alignment for selected Aptx homologs. Residue classifications are marked in the legend. AOA1 disease variants are shown in red sequence under the alignment.



Supplementary Fig. 6 Ap4A hydrolase activity of Aptx mutants. Release of AMP (relative to full length wild type Aptx-FL) from AP4A was measured and error bars reflect standard deviation of three independent measurements.

Supplementary Movie Legends

Supplementary Movie 1. **Aptx engagement of a nicked or gapped DNA-adenylate.** Aptx employs the helical wedge with Phe34 (Blue) to displace a stacked 5'-adenylate (orange). The DNA (green) morphs between a model B-DNA conformation and the backbone conformation observed in the Aptx–DNA–AMP–Zn complex structure. A slight under-winding of the duplex is observed upon binding. Grey DNA is a modeled conformation of the predicted positioning of the upstream region of nick or gapped duplex bearing a 5'-AMP. Intercalation of the wedge helix into the base stack necessitates displacement of the upstream DNA. HIT domain is shown in purple and Znf in gold/brown.