Supplementary Information

Structural basis for duplex RNA recognition and cleavage by *A. fulgidus* C3PO

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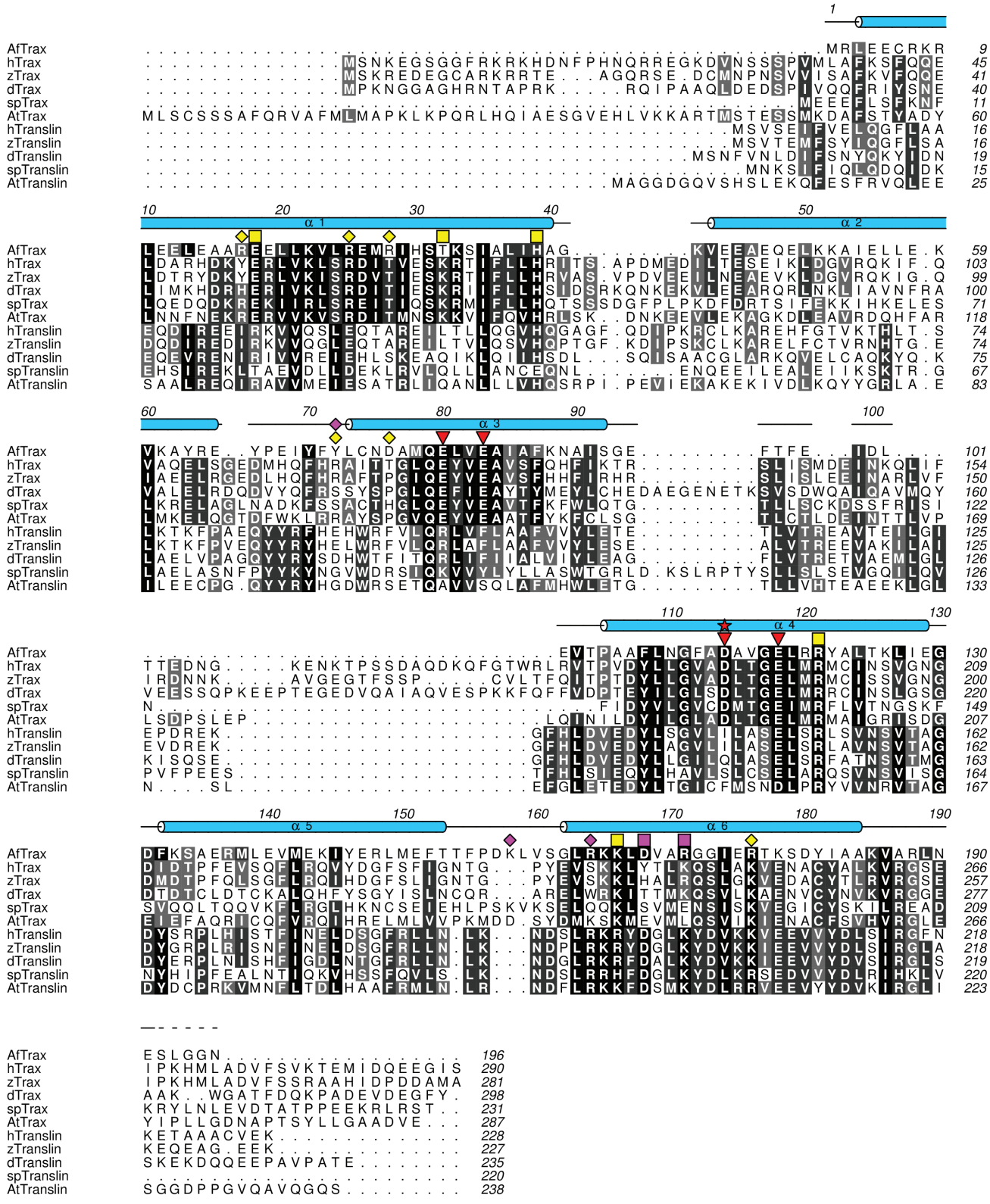
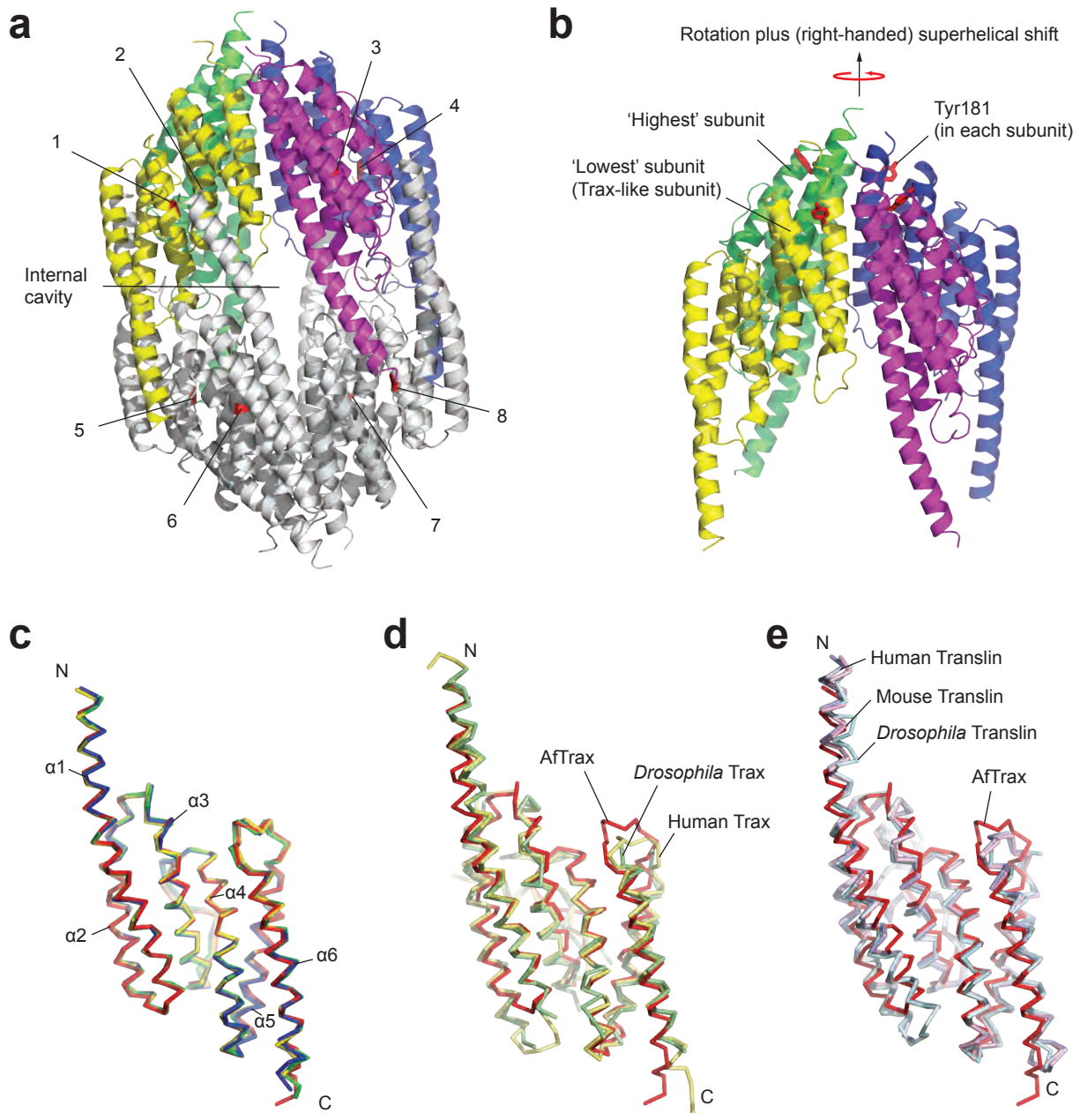
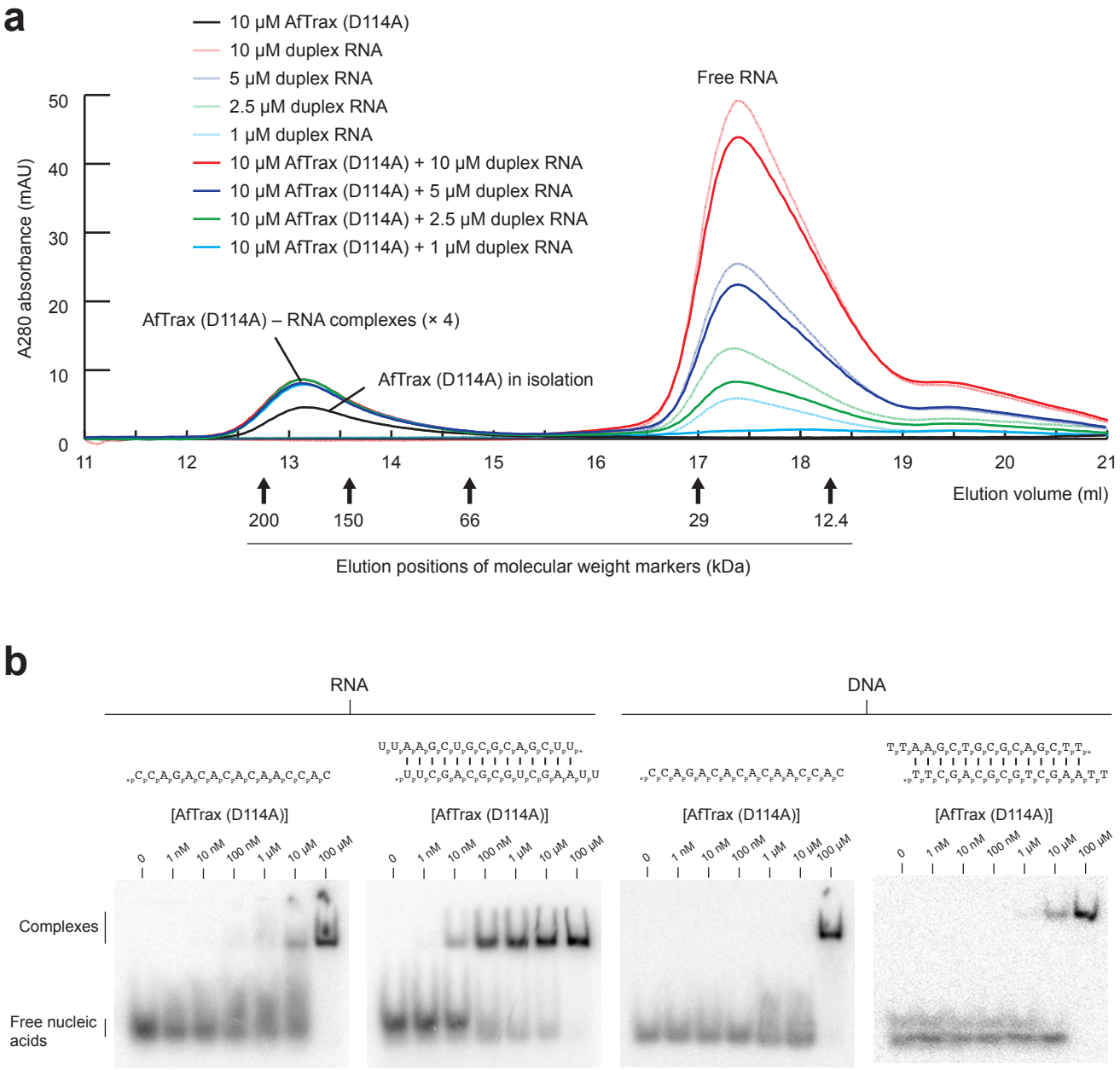


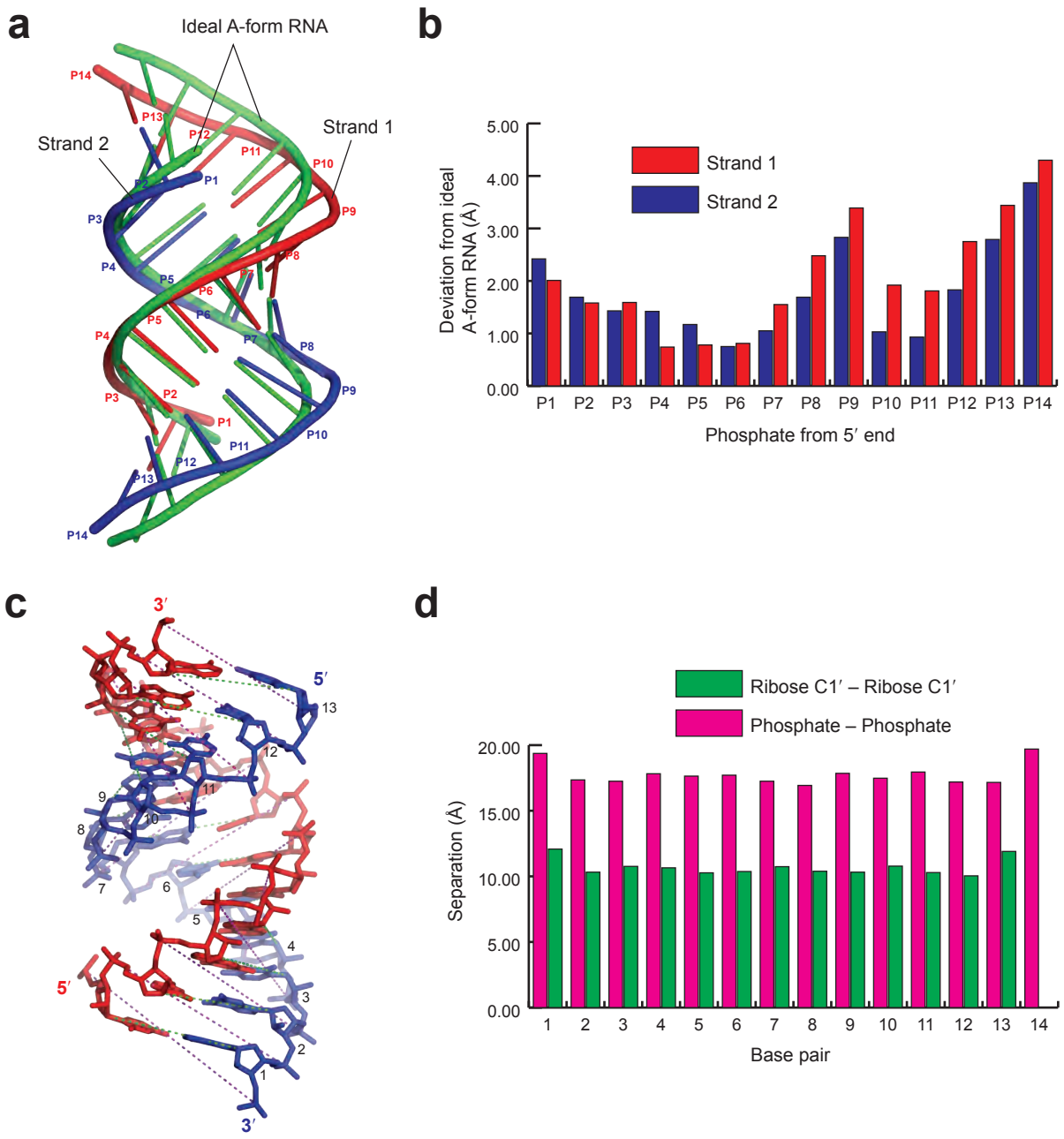
Supplementary Figure 1. Structure-based sequence alignment of AfTrax and representative eukaryotic Trax and Translin proteins. AfTrax secondary structure (helices α1 – α6 and loops) and sequence numbering are shown across the top. Dotted regions are absent from the structure. Red downward triangles indicate conserved catalytic glutamate or aspartate residues. The residue mutated in AfTrax-mut (D114A) is highlighted by a red star. Diamonds indicate RNA-interacting residues; squares indicate residues whose side chains interact with RNA-interacting residues. Yellow (diamonds or squares) indicates residues from Trax-like subunits, whereas purple indicates residues from Translin-like subunits. The eukaryotic sequences are from humans (h), zebrafish (z), *Drosophila* (d), *S. pombe* (sp) and *A. thaliana* (At). Shading shows sequence conservation in either the Trax or Translin subfamily (both including the AfTrax sequence.) The figure was formatted using *ALINE*[54].



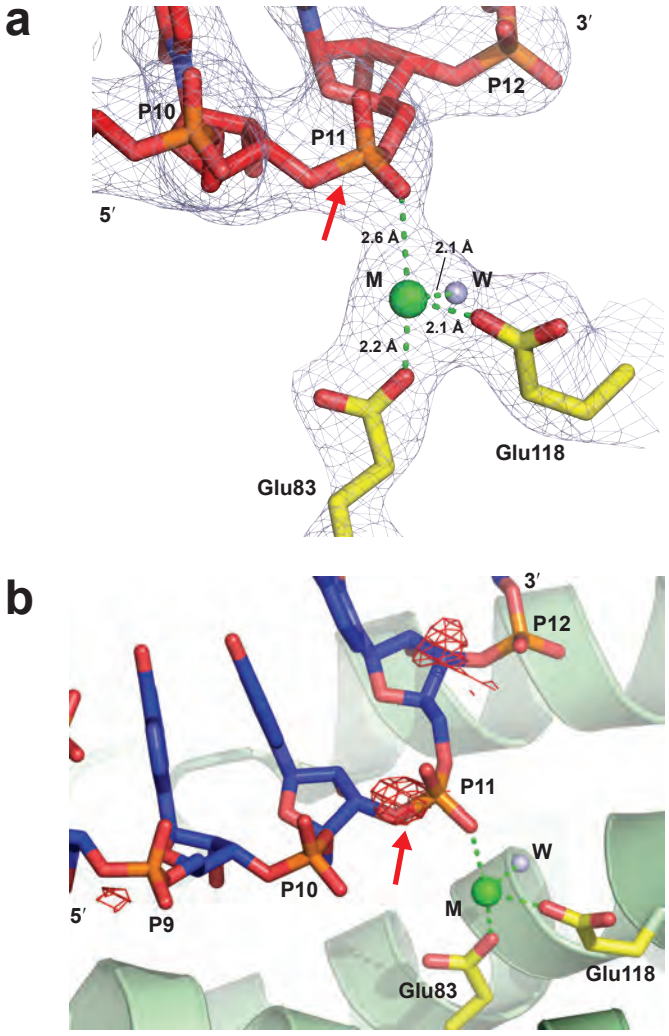
Supplementary Figure 2. AfC3PO octamer and monomer structure. (**a**) Octameric subunit arrangement in AfC3PO. In this view of the apo structure, the four subunits of the 'upper' tetramer are colored yellow, green, blue and magenta and the four subunits of the 'lower' tetramer, in a similar but inverted arrangement, are colored uniformly grey. 'Catalytic' Glu83 residues from each of the eight subunits are colored red and labeled 1 – 8. (**b**) Superhelical subunit arrangement in AfC3PO. Subunits in the upper and lower tetramers are related by a ~ 90° rotation and a small right-handed superhelical shift along the rotation axis. This creates a shallow spiral arrangement, with one subunit (yellow) adopting the lowest position in the spiral and another (shown in green) the highest. Tyr181 side chains are depicted (red) to illustrate the relative shift along the rotation axis. (**c**) Superposition of the four (independent) chains that make up either the upper or lower AfC3PO tetrameric unit, colored red, yellow, green and blue. Inter-subunit Cα root mean square deviations (rmsds) are 0.80 – 0.89 Å. α helices α1 – α6 of the fold are labeled. (**d**) Superposition of a representative AfTrax monomer with Trax from humans[17] (3PJA/J, rmsd 1.95 Å) and *Drosophila*[30] (3RIU/C, rmsd 2.32 Å). (**e**) Superposition of a representative AfTrax monomer with Translin from humans[17] (3PJA/A, rmsd 2.31 Å), mouse[31] (1KEY/A, rmsd 2.42 Å) and *Drosophila*[30] (3RIU/A, rmsd 2.45 Å).



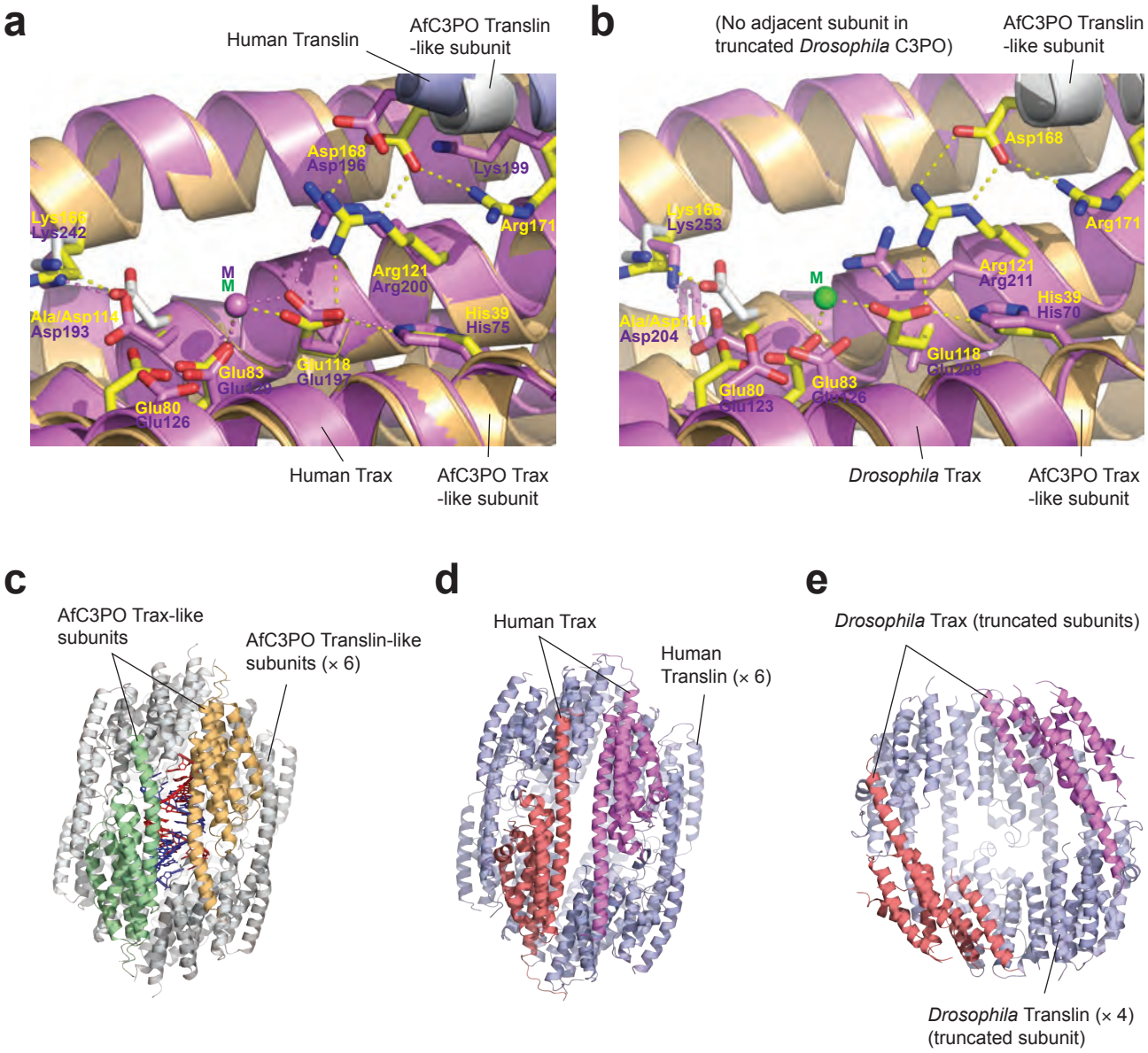
Supplementary Figure 3. Interactions of AfTrax (D114A) with nucleic acids. (**a**) Analytical gel filtration profiles of AfTrax (D114A) in isolation and in complex with various concentrations of 14 bp siRNA-like RNA duplex (duplex as crystallized). The AfTrax concentration (10 μM) refers to the AfTrax monomer concentration. The elution positions of standard molecular weight markers are shown across the bottom. Wild type AfTrax elutes at the same position as AfTrax (D114A) (not shown). (**b**) Native gel mobility shift assays showing AfTrax (D114A) association with 5′ labelled (*) 16 mer RNA and DNA, single-stranded and double-stranded.



Supplementary Figure 4. Conformation of duplex RNA bound inside AfC3PO. (**a**) Superposition of an ideal A-form RNA duplex (green) with the RNA duplex bound inside AfC3PO (strands red and blue; cartoon representation). Phosphate groups (P1 – P14) are labeled from the 5′ ends. (**b**) Chart depicting deviation from A-form structure for the phosphate groups on the two strands. (**c**) View of the duplex illustrating inter-strand phosphate and ribose C1′ distances (dotted pink and green respectively). Base pairs are numbered 1 – 13 from one end. (**d**) Chart depicting inter-strand distances along the duplex. Base pairs are numbered as in panel **c**. Terminal phosphate groups (1 and 14) and base pairs (1 and 13) show increased separations.



Supplementary Figure 5. Electron density at the AfC3PO Trax-like subunit catalytic sites. (**a**) $2F_O - F_C$ electron density contoured at 1.0 σ surrounding the substrate RNA, catalytic metal (M) and coordinating conserved catalytic glutamate side chains. W is a coordinated water. The putative scissile bond is indicated by a red arrow. Metal coordination distances are shown. (**b**) $F_O - F_C$ difference density contoured at –3.0 σ around one strand of the substrate RNA and a Trax-like subunit catalytic site. The putative scissile bond is indicated by a red arrow.



Supplementary Figure 6. Comparisons of AfC3PO, human C3PO and 'truncated' *Drosophila* C3PO. (**a**) Superposition of an AfC3PO Trax-like subunit catalytic site (RNA omitted) with a Trax catalytic site from human C3PO[17] (PDB code 3QB5, chains K and C). AfC3PO subunits are colored orange and grey, with yellow side chains, and human subunits are colored purple (Trax) and blue (Translin) with purple side chains. A manganese ion (M, purple sphere) soaked into crystals of human C3PO occupies the same coordinated position as the magnesium ion (M, green sphere behind) in the RNA complex of AfC3PO. (**b**) Superposition of an AfC3PO Trax-like subunit catalytic site (RNA omitted) with a Trax catalytic site from 'truncated' *Drosophila* C3PO[30] (PDB code 3RIU, chain C), formed from truncated Trax and Translin subunits. Coloring is as in panel **a**, with *Drosophila* replacing human. There is no similar adjacent Translin subunit in the truncated *Drosophila* C3PO crystal structure. (**c**) AfC3PO octamer with bound RNA. (**d**) Human C3PO octamer (PDB code 3PJA)[17] composed of two Trax subunits (salmon and violet) and six Translin subunits (blue). (**e**) 'Truncated' *Drosophila* C3PO hexamer (PDB code 3RIU)[30] composed of two truncated Trax subunits (salmon and violet) and four truncated Translin subunits (blue).