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Supplementary Materials for

Unraveling self-assembly pathways of the 468-kDa proteolytic machine TET2

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This PDF file includes:

Supplementary Materials

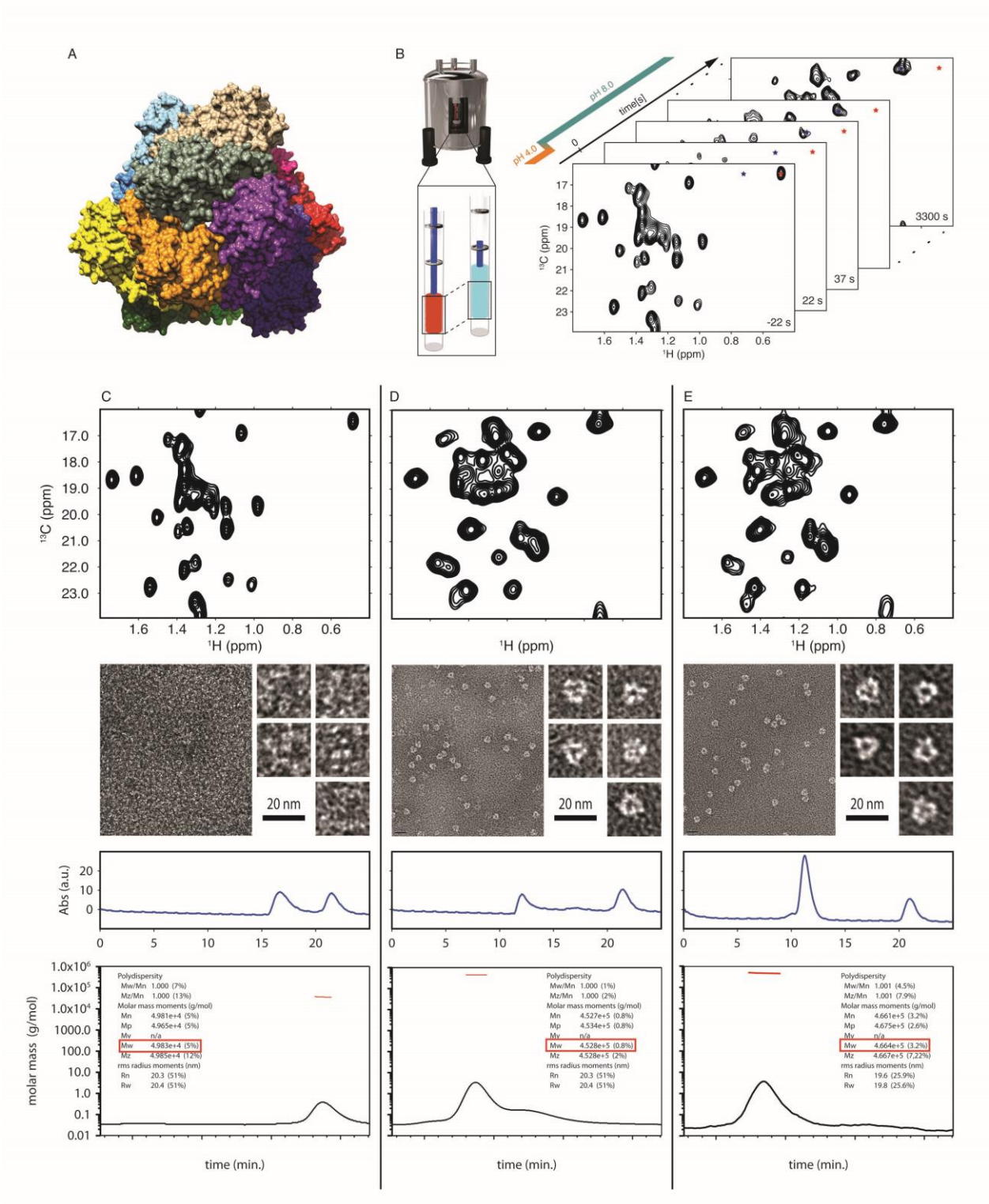

fig. S1. TET2 structure, schematics of the NMR in situ kinetics experiment and disassembly, and re-assembly of TET2. Surface representation of TET2 structure viewed from the apex of the tetrahedron (**A**), each subunit is represented by different colors. (**B**) In situ stopped flow pH jump (t=0 s) and schematic of real-time NMR experiment showing selected spectra from the series acquired during self-assembly. The first spectrum of the series (t=-22 s) corresponds to acid stabilized monomer (pH=4) before self-assembly initiation (pH=8). The red star marks A194 peak of monomer stabilized at pH 4, which disappears upon self-assembly initiation. The blue star marks A194 peak of dodecamer, which increases in intensity during the course of self-assembly. (**C**, **D**, **E**) ^{1}H-^{13}C SOFAST-Methyl-TROSY spectra, representative EM snapshots, SEC-MALLS and gel filtration profiles of disassembled TET2 stabilized in low pH (C), re-assembled TET2 after jump to neutral pH (D), and native TET2 before disassembly (E). Molecular masses were determined by MALLS analysis. Molecular mass elution profiles are shown as a red horizontal bars over the peaks. MALLS determined molecular masses are highlighted in red squares in the output reports. The mass of the monomer is slightly higher (49 kDa) than theoretical (39 kDa). However, the size of particles in the EM is at the limit of the EM resolution, which is compatible with a monomer or a dimer. The slight overestimation of molecular mass in disassembled sample could originate from the presence of monomer in equilibrium with small population of dimers.

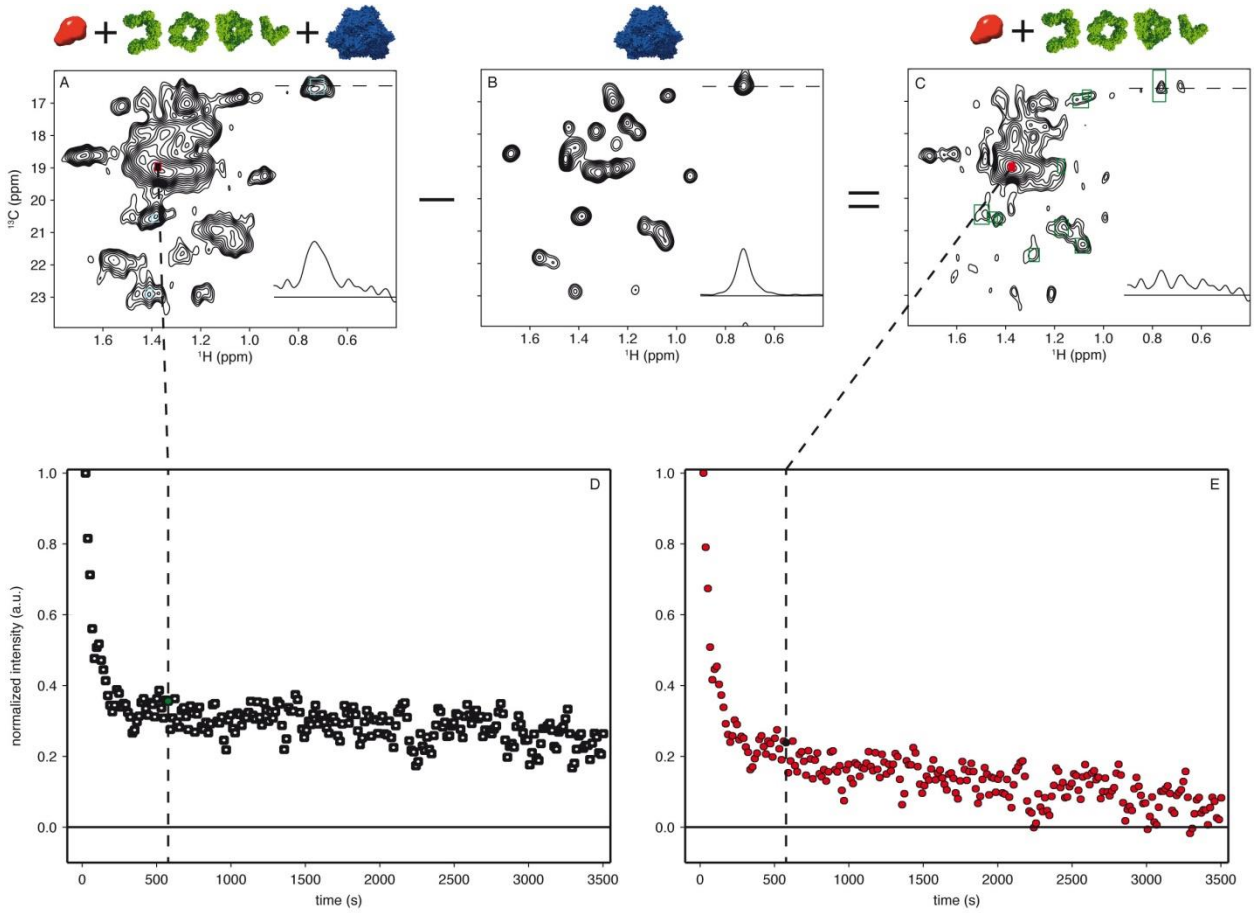

fig. S2. Subtraction of the dodecamer evolution from the kinetic spectral series for deconvolution of the flexible monomeric intermediate evolution from the dodecamer buildup. Traces in NMR spectra show ^{1}H 1D projections at position of ^{13}C resonance of dodecamer A194. (**A**) Typical spectrum before dodecamer subtraction. The rectangles delineate the integration area for the combined build-up of the oligomeric intermediate and dodecamer (cyan) and the flexible intermediate before subtraction (red). (**B**) Scaled spectrum of the dodecamer. (**C**) A dodecamer subtracted spectrum including areas for the oligomeric intermediate (green rectangles) and the flexible intermediate after the dodecamer subtraction (red rectangle). Note the increased linewidth in the NMR spectrum during the self-assembly time-course (off-equilibrium) (A) in comparison to NMR spectrum of reassembled TET2 (B). (**D**) and (**E**) time evolution of flexible intermediate intensity before and after dodecamer subtraction, respectively. The vertical dashed lines mark the spectrum time-point used in the example. Before the subtraction (D) the intensity of the flexible intermediate does not decay to zero in comparison to dodecamer subtracted flexible monomer evolution.

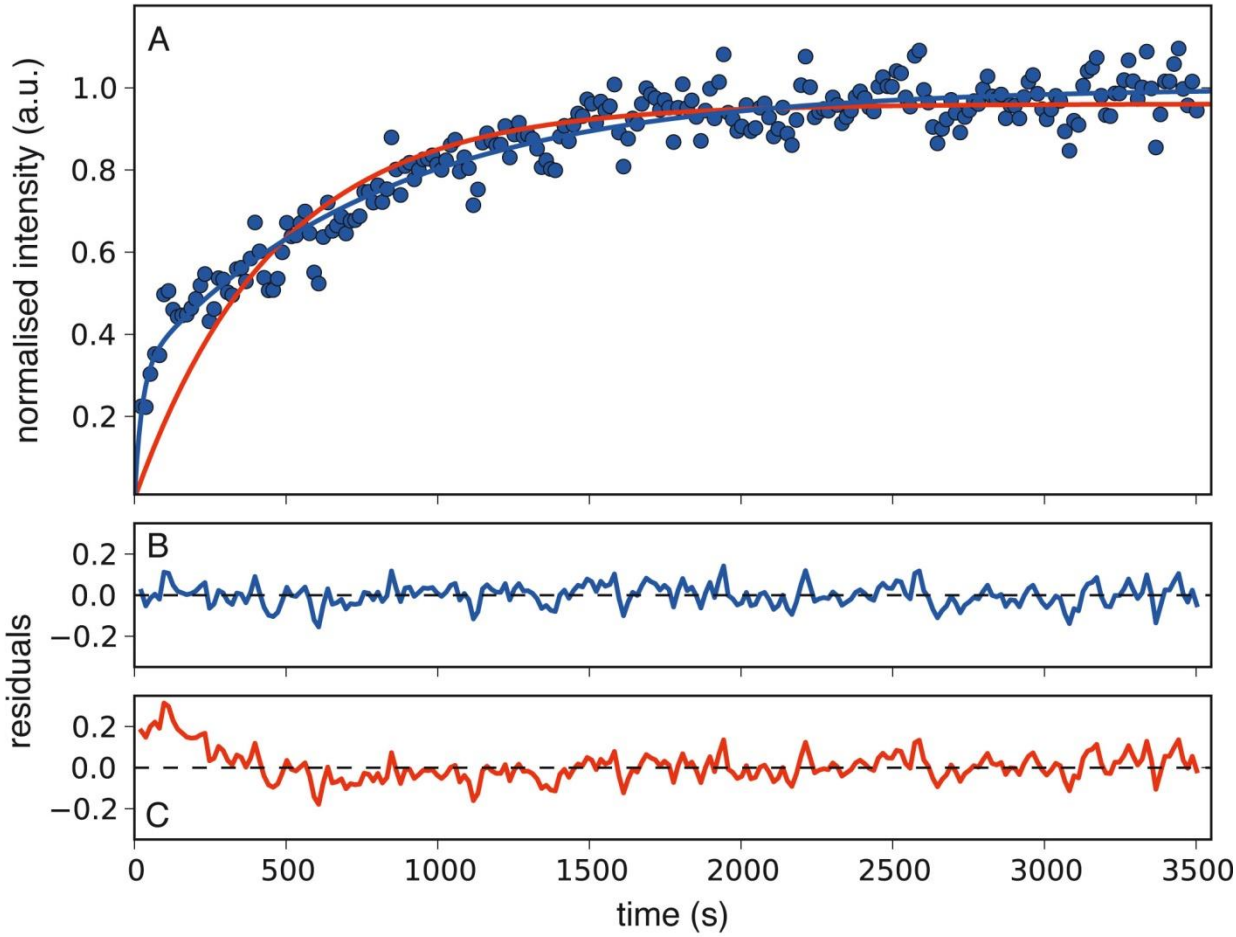

fig. S3. Fit of dodecamer and dodecamer-like species buildup curve. (**A**) Double (blue line) and single (red line) exponential fit of dodecamer/dodecamer-like specie evolution used for the subtraction of the dodecamer build from kinetic spectra series. (**B**) and (**C**) residuals of double and single exponential fit, respectively.

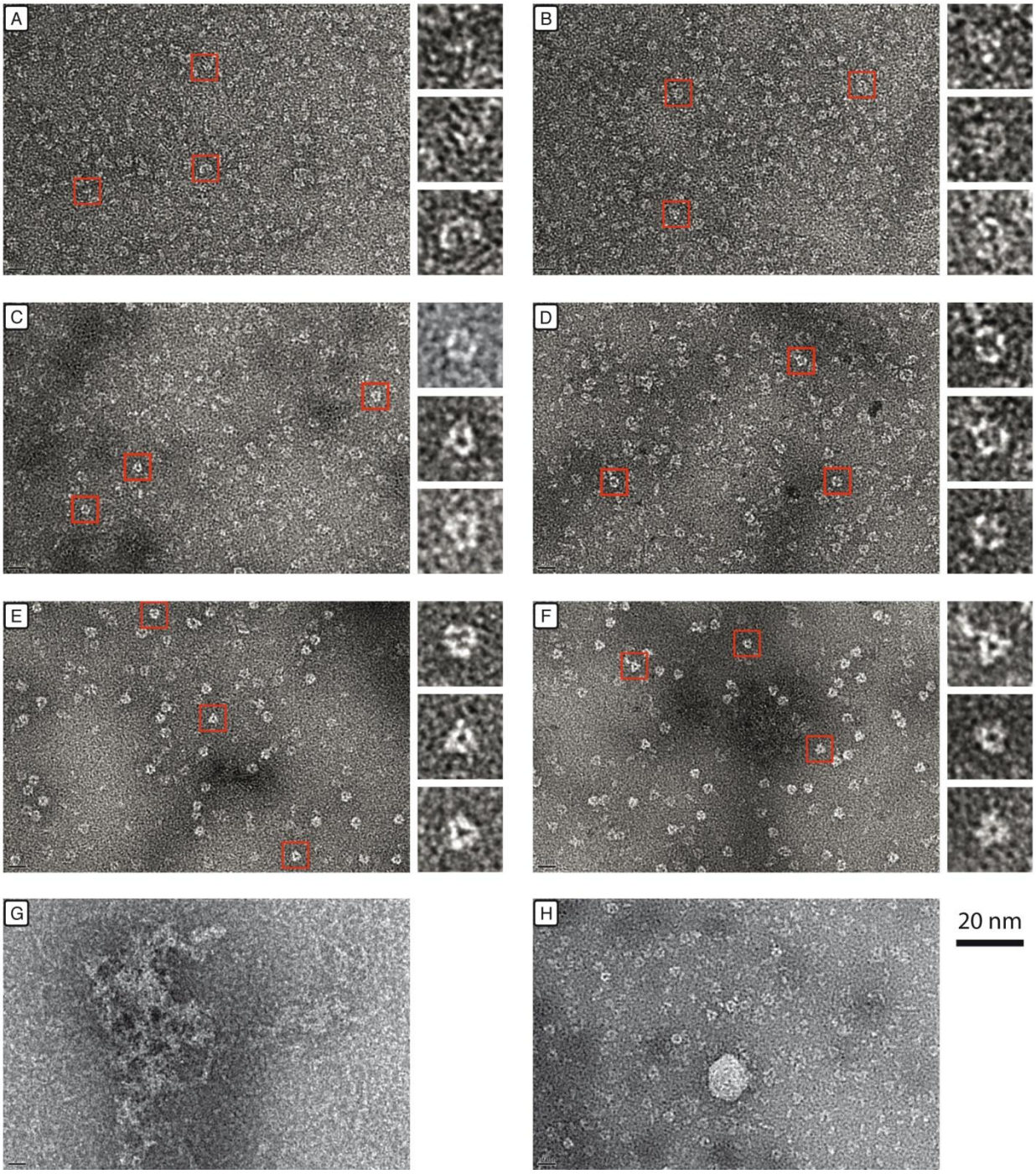

fig. S4. EM images of TET2 self-assembly including the zooms of the red-squared regions. (**A**, **B**) EM images of self-assembly reaction stained at 30 s after initiation of oligomerization. (**C**, **D**) EM images of self-assembly reaction stained at 120 s. The zoomed regions show representative oligomeric intermediates. (**E**, **F**) EM images of the self-assembled TET2 with zooms showing different projections of the tetrahedron like quaternary structure of the TET2.

(**G**) EM image of soluble aggregates present in low pH stabilized monomer and (**H**) aggregates of associated intermediates from the course of the self-assembly.

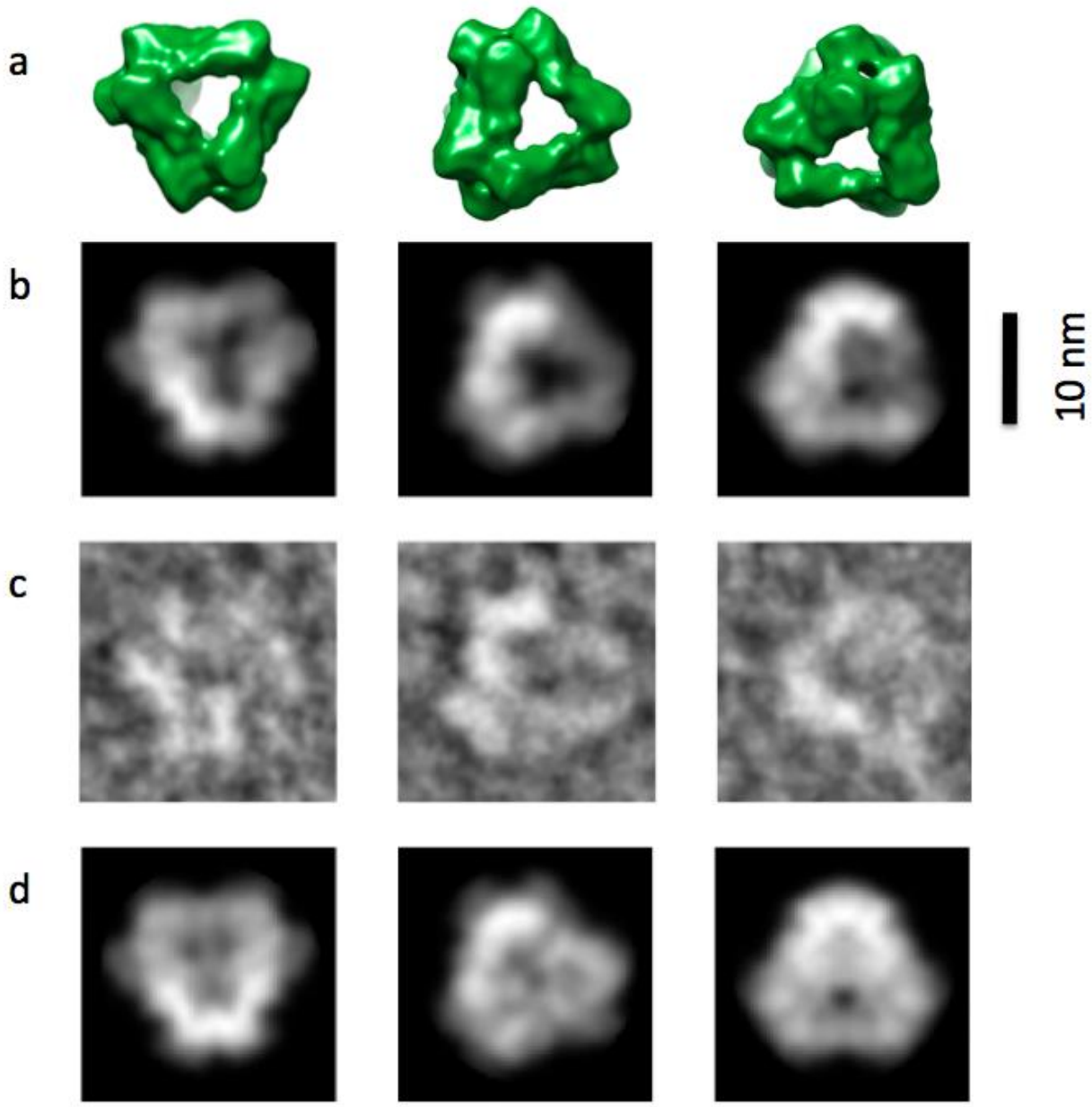

fig. S5. EM images of TET2 decamer intermediate. (**A**) PDB based model of a decamer filtered to 20 Å resolution viewed in different orientations. (**B**) 2D projections of the 20 Å filtered decamer model in the same orientation as in row A. (**C**) Negative staining image of molecule corresponding to decamer seen in the same orientation as in row A and B. (**D**) For comparison: 2D projections of the 20 Å filtered dodecamer model in the same orientation as in row A and B.

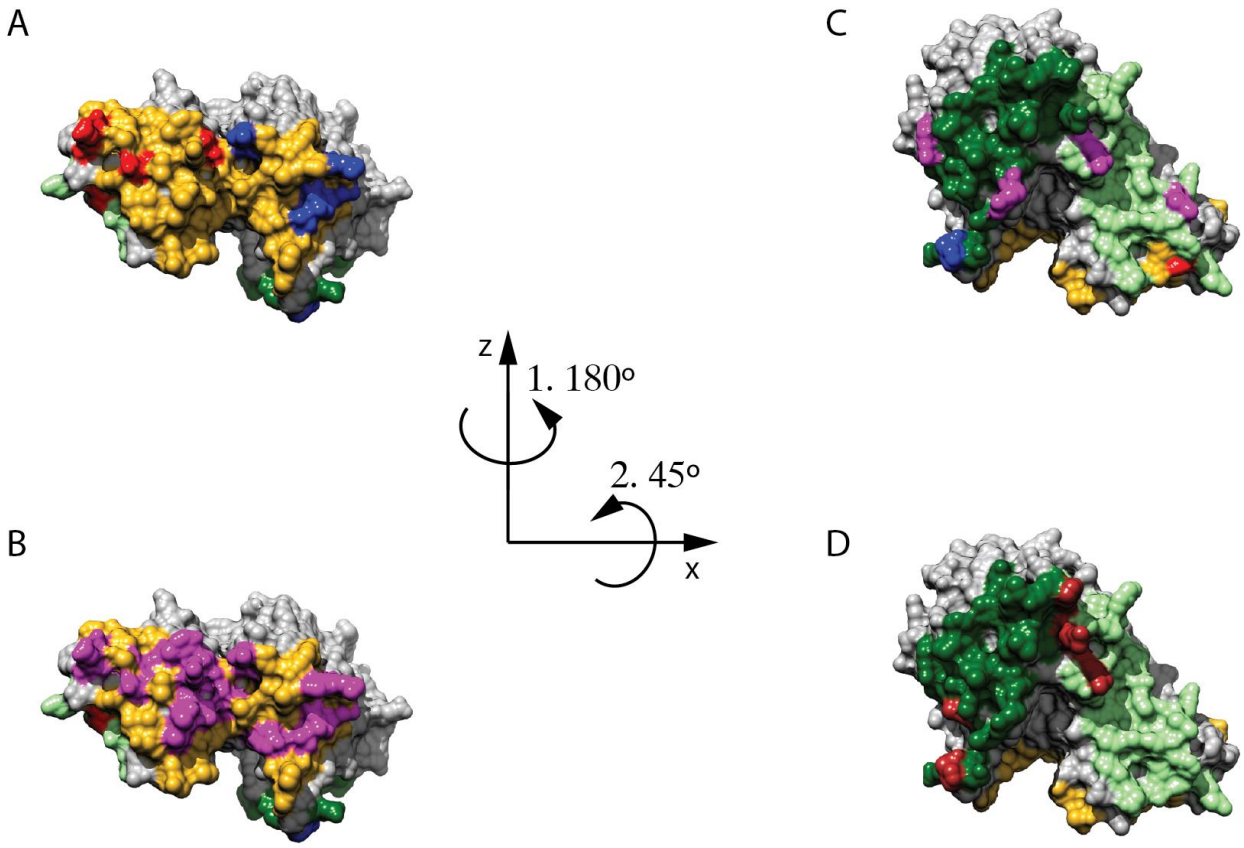

fig. S6. Analysis of TET2 oligomeric interfaces using PISA (*45*). Yellow dimerization interface with positive (blue) and negative (red) residues participating in salt bridges presented in (**A**) and hydrogen bonds (magenta) in (**B**). The two trimerization inerfaces are shown in dark and light green. The positive and negative residues involved in salt bridges are colored in blue and red, respectively, while residues involved in hydrogen bonds are colored in magenta (**C**). The residues of trimerization interfaces mutated in Franzetti et al. (2013) (*13*) are colored in red (**D**).