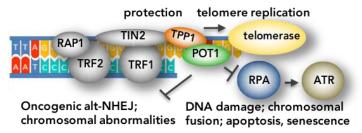
Title: Structure of the POT1-TPP1 telomere end-binding complex.

## **Supporting Material**



**Fig 1.** The multiple and diverse roles of the POT1-TPP1 complex in telomere maintenance. The complex binds exclusively to telomere DNA to prevent induction of DNA damage signaling. It also resolves DNA structure and regulates telomerase activity.



**Fig 2.** Purified POT1-TPP1 complex. SDS-PAGE gel of an assembled POT1-TPP1-ssDNA complex after affinity pull-down and size-exclusion chromatography. The upper band on the left represents the 70 kDa POT1 protein and the lower band represents the 30 kDa TPP1 protein. The right lane is a molecular weight standard marker.

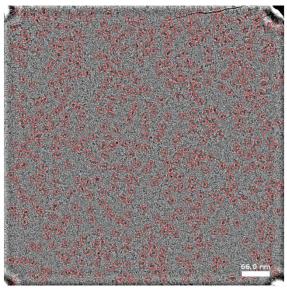
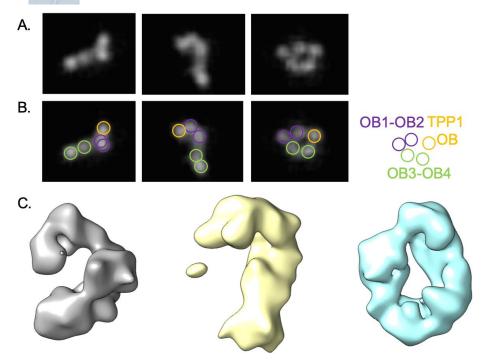


Fig 3. Cryo-EM micrograph of purified POT1-TPP1-DNA complex on graphene-oxide coated UltraAu foil grid. Magnification is 62,000 X. Particles are identified with red circle that has a 160 Å diameter.



The POT1-TPP1-ssDNA Fig complex is dynamic and adopts multiple conformations. Representative 2D averages for picked particles reveals multiple different shapes. The five individual OB-fold domains of POT1 and TPP1 can be seen individually as "beads-on-astring". B. Circles overlaid on the 2D averages shown in panel A depict individual OB-fold domains predicted regions of the complex. The key on the right is color-coded for the individual domains. C. dimensional reconstructions of POT1-TPP1-DNA complexes reveals the intrinsic dynamics of the assembled nucleoprotein complex. The three reconstructions were sorted from the same dataset and are resolved at ~13 À resolution.

## LITERATURE CITED:

- R. K. Moyzis et al., A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. Proc Natl Acad Sci U S A 85, 6622-6626 (1988).
- 2. J. Nandakumar, T. R. Cech, Finding the end: recruitment of telomerase to telomeres. Nat Rev Mol Cell Biol 14, 69-82 (2013).
- 3. E. H. Blackburn, K. Collins, Telomerase: an RNP enzyme synthesizes DNA. Cold Spring Harb Perspect Biol 3, (2011).
- 4. T. de Lange, Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev 19, 2100-2110 (2005).
- 5. E. L. Denchi, T. de Lange, Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. Nature 448, 1068-1071 (2007).
- 6. X. Guo et al., Dysfunctional telomeres activate an ATM-ĀTR-dependent DNA damage response to suppress tumorigenesis. EMBO J 26, 4709-4719 (2007).
- B. A. Moser, L. Subramanian, L. Khair, Y. T. Chang, T. M. Nakamura, Fission yeast Tel1(ATM) and Rad3(ATR) promote telomere protection and telomerase recruitment. PLoS Genet 5, e1000622 (2009).
- B. P. Baumann, T. R. Cech, Pot1, the putative telomere end-binding protein in fission yeast and humans. Science 292, 1171-1175 (2001).
- 9. H. Xin et al., TPP1 is a homologue of ciliate TEBP-beta and interacts with POT1 to recruit telomerase. Nature 445, 559-562 (2007).
- 10. F. Wang *et al.*, The POT1-TPP1 telomere complex is a telomerase processivity factor. *Nature* **445**, 506-510 (2007).
- 11. J. Nandakumar, E. R. Podell, T. R. Cech, How telomeric protein POT1 avoids RNA to achieve specificity for single-stranded DNA. *Proc Natl Acad Sci U S A* **107**, 651-656 (2010).
- 12. M. Lei, E. R. Podell, T. R. Cech, Structure of human POT1 bound to telomeric single-stranded DNA provides a model for chromosome end-protection. *Nat Struct Mol Biol* 11, 1223-1229 (2004).
- 13. C. Rice et al., Structural and functional analysis of the human POT1-TPP1 telomeric complex. Nature communications 8, 14928 (2017).
- 14. C. Chen et al., Structural insights into POT1-TPP1 interaction and POT1 C-terminal mutations in human cancer. Nature communications 8, 14929 (2017).