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TRAF-specific Zinc Finger domain following RING domain in TRAF6 induces helix-formation of the interdomain linker and dimerization

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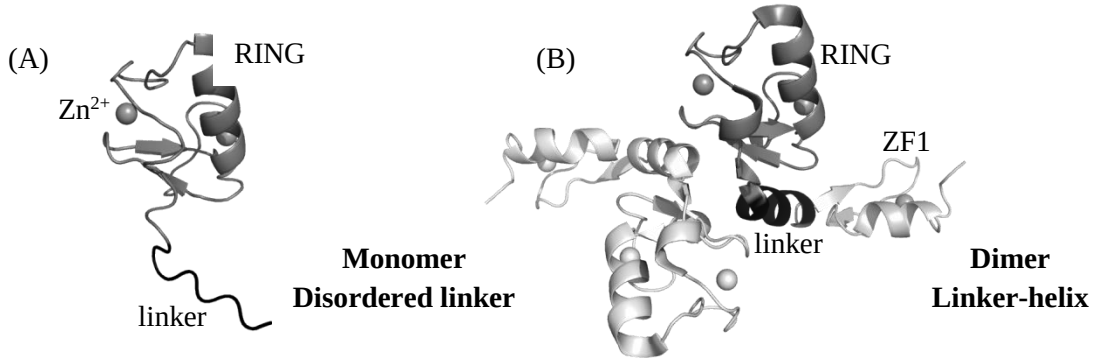
Tumor necrosis factor Receptor Associated Factor-6 (TRAF6) is a K63-polyubiquitin E3 ligase involved in the activation of NFκB. TRAF6 consists of the N-terminal RING domain and four zinc fingers, and the C-terminal coiled coil and TRAF-C domain. It has been known that TRAF6 promotes its auto-ubiquitination-activity by the dimerization of the N-terminal region which includes RING domain and zinc fingers, at least 1st zinc finger (ZF1). Although its dimerization requires not only RING domain but also ZF1, ZF1 is not involved in the dimer-interface. Instead, the linker between RING domain and ZF1 adopts the helical structure (linker-helix) and forms the dimer-interface together with the RING domain. However, the mechanism for the helix-formation of the interdomain linker and the resultant dimerization are still unclear. In fact, the construct for RING domain and the linker region without ZF1 did not form the linker-helix and the resultant dimer formation anymore.

Here, we prepared the isolated RING domain (R), the RING and ZF1 region-constructs (RZ_1), and the RING and ZF1-3 region-constructs ($RZ_{1\text{-}3}$), and performed size exclusion chromatography, dynamic light scattering, chemical cross-linking, and NMR experiments using these constructs. Our results suggested that R even showed the weak dimerization-ability and the attachment of ZF1 enhanced the dimerization-ability accompanied with the helix-formation of the linker in solution. Eventually, since the auto-ubiquitination site, K124, positions in the linker helix, RZ_1 can be used as a screening target to find a new anti-inflammatory drug related to NF-κB signaling pathway activated by TRAF6.



Figures ZF1 induced the helical formation for the linker between RING and ZF1 and dimerization. RING domain and the linker region without ZF1 (PDBID: 2ECI) (A), and with ZF1 (PDBID: 3HCS) (B).