Cryo-EM structures reveal dramatic remodeling of the p97 hexamer by the multi-domain adapter UBXD1

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P97/VCP is an essential AAA+ molecular machine critical for many cellular processes. While the established function of p97 is the ATP-dependent threading of proteins through its central pore, this activity is regulated by adapter proteins that endow critical functions in substrate specificity, cellular localization, and specific enzymatic functions, for example removal of ubiquitin molecules from canonical p97 clients. UBXD1 is a poorly-characterized p97 adapter, and is implicated in p97's roles in autophagic clearance of damaged mitochondria and lysosomes. While UBXD1 binding has been demonstrated to be impaired by disease-causing p97 mutations, indicating its relevance in maintaining cellular homeostasis, details regarding how UBXD1 alters p97 function in the context of the full-length proteins have remained elusive. To better understand how UBXD1 affects p97 function, we determined high-resolution cryo-EM structures of p97-UBXD1 complexes, which reveal that a single UBXD1 molecule disrupts normal p97 protomer-protomer interactions using both annotated and previously uncharacterized p97-interacting domains. An additional state reveals a striking UBXD1-mediated hexamer ring opening, in which contacts between neighboring p97 protomers are completely disrupted. Three-dimensional variability analysis reveals that these structures represent snapshots in a continuous spectrum of p97 ring opening, lending support to the proposed mechanism of UBXD1-dependent p97 remodeling. These results, coupled with biochemical analyses, provide the most comprehensive information to date on the molecular basis for UBXD1's effects on p97 structure and function, and reveal potential roles in regulating p97 substrate processing activity.