CryoEM structure of a multivalent ubiquitin ligase complex

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Protein ubiquitination is a common posttranslational modification with central roles in eukaryotic cellular physiology. The selection of targets for modification is largely determined by the E3 ubiquitin ligases, which catalyze the transfer of ubiquitin by positioning substrates next to activated E2~ubiquitin conjugates. Of the over 600 known E3 ubiquitin ligases, the largest subclass are the Cullin3-Ring-Ligases (CRL3) with over 70 members. CRL3s are modular assemblies that involve multiple components, including BTB domain substrate binders. These binders usually combine a N-terminal BTB Cul3-binding domain and a C-terminal substrate-binding domain within a single polypeptide. Notably, BTB domains can self-associate into stable dimers, pentamers and oligomers and thus drive the multimerization of CRL3 complexes.

We have identified interactions between KCTD5, a pentameric CRL3 BTB adaptor protein, and several G-protein heterodimers. This raises the possibility that members of the CRL3 E3 ligase family regulate G-protein signalling by targeted ubiquitination. We demonstrate the direct, non-exclusive binding of both Gßγ heterodimers and the Cul3 N-terminal domain with KCTD5 and determined the cryo-EM structure of a 560 kDa 5:5:5 KCTD5:Gßγ:Cul3 complex to a resolution of 3.0 Å resolution. The 15-chain assembly has pseudo-C5 symmetry with large scale dynamics involving rotations of over 40° between the KCTD5/Gßγ and KCTD5/Cul3 moieties of the complex. Modeling a full-length Cul3/Rbx1/E2~ubiquitin assembly into the complex reveals that one particular rotamer positions Gßγ within ~5 Å of the E2~Ub thioester bond. Previously described E3/substrate structures were monovalent and involved flexible peptide substrates. The KCTD5/Gßγ/Cul3 complex presented here demonstrates the role of multivalency in the CRL3 ligases and reveals how the architecture of an E3 ligase can position a structured target for ubiquitination.