

P002 E3-independent self-ubiquitination of Ub-binding proteins
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Ub-binding proteins are crucial mediators and regulators of Ub-dependent cellular processes such as protein degradation, DNA repair, membrane trafficking and others. Up to date, close to 20 families of Ub-binding domains (UBDs) have been described and shown to distinguish between different types of ubiquitination. Besides mediating the contact to ubiquitinated targets most UBDs trigger the monoubiquitination of the host protein. This process is called 'coupled monoubiquitination' and leads to an intramolecular UBD/Ub interaction, which functionally inactivates the protein. By investigating the molecular mechanisms underlying coupled monoubiquitination we have discovered that UBD-containing proteins undergo E3-independent self-ubiquitination. Ub-loaded E2 enzymes can directly cooperate with UBDs of different types (UBA, UIM, VHS, UBZ, UBM, NFZ) and transfer Ub to a substrate lysine. Using a protein replacement approach as well as FRET technology we verified that E2-enzymes mediate the monoubiquitination of UBD-proteins in cells independently of E3 ligases. We propose that E2-mediated inactivation of Ub-binding proteins represents an active homeostatic mechanism responsible for inhibition of non-physiological interactions with ubiquitinated substrates.