

Abstract

Ubiquitination is one of the most abundant and widespread post-translational modifications in biology. Modification of a protein with the addition of one or several ubiquitin moieties alters the structure, activity or interaction of the modified protein with effector proteins. Ubiquitination is involved in the proteasomal degradation of damaged, misfolded or unneeded proteins as well as the DNA damage response, protein trafficking and signal transduction. Ubiquitin is canonically attached to lysines of a target protein by forming an isopeptide bond through its C-terminus. It has the potential to form isopeptide bond with already attached ubiquitin units forming chains of ubiquitin, linked through one of several positions in the initial ubiquitin protein. This modification is the result of the concerted efforts of a triad of enzymes, the E1, E2 and E3 enzymes. Proteins containing ubiquitin-binding domains are able to recognize and bind the ubiquitin modification and effectuate the proper cellular response, e.g. ubiquitin linked through one specific lysine generally confer its transport to the proteasome complex where it is degraded. Dysfunctional components within this signaling system can lead aberrant behavior of the cell and potentially lead to diseases in the systemic context of an organism, e.g. dysregulation of the E3 ligase MG53 can affect the cellular response to insulin and contribute to generation of diabetes in the organism.

Understanding the function and dysfunction of ubiquitin signaling is crucial for developing clinical therapeutics to mitigate aberrant ubiquitin signaling. Mass spectrometry based quantitative proteomics combined with advanced purification techniques to probe the ubiquitinome has advanced our understanding of ubiquitin signaling immensely over the last decade, however, techniques to investigate specific ubiquitin chains are still lacking.

The work presented in this thesis aims to establish novel ubiquitin enrichment methods that allow in-depth analysis of the ubiquitinome at the proteins level as well as the peptide level. This versatile approach is based on RNA interference-mediated exchange of endogenous ubiquitin with a tagged recombinant analog. The published StUbEx PLUS method introduces a recombinant ubiquitin with an internal tag that allows specific non-antibody enrichment of peptides containing ubiquitin sites for global mapping of ubiquitin site. This method is further modified in the StUbEx PLUS-MINUS method where the recombinant ubiquitin can be modified to target specific types of ubiquitination signaling, filling a gap in the ubiquitin-analysis toolbox. Applying the method, we investigate the role of some of the less understood ubiquitin chains.