OUTLINE OF THE THESIS

In **Chapter 1**, I review SUMOylation in general, with a focus on mass spectrometry, and describe current SUMO proteomics methodology in addition to technical hurdles that hamper efficient study of SUMO by mass spectrometry.

In **Chapter 2**, we investigate SUMOylation dynamics in response to the DNA damaging agent methyl methanesulfonate (MMS). We uncover hundreds of SUMOylated proteins, of which a subset of functionally related proteins are differentially SUMOylated in response to MMS. We find a pair of histone demethylases, JARID1B and JARID1C, to be inversely regulated by SUMO, despite their functional and structural similarity.

In **Chapter 3**, we perform a screen for direct interaction partners of the SUMO-targeted ubiquitin E3 ligase RNF4. We identify the ubiquitin protease USP11 as an interactor of RNF4, and investigate the relationship between RNF4, USP11, and PML. We find USP11 to be a SUMO-targeted ubiquitin protease, with the ability to counteract the function of RNF4.

In **Chapter 4**, we develop a method for studying SUMO at the site-specific level. Through use of a lysine-deficient SUMO mutant bearing an additional carboxyl-terminal arginine, we succeed in identification of 103 SUMOylated lysines in endogenous proteins. Study of these SUMO sites reveals an inverted SUMO motif and a hydrophobic cluster SUMO motif.

In **Chapter 5**, we combine lessons learned in Chapters 2 and 4 in order to study global SUMOylation dynamics throughout cell cycle progression. From a set of over 500 confirmed SUMOylated proteins, we find over 100 to be differentially SUMOylated over the cell cycle, and additionally pinpoint 202 SUMOylated lysines.

In **Chapter 6**, we optimize and extrapolate the lysine-deficient SUMO site identification methodology, and successfully identify 3,246 SUMOylated lysines in 1,364 proteins. Through thorough bioinformatical analyses, we shed new light on the SUMO proteome, and discover many functionally related proteins and entire protein complexes to be targets for SUMOylation. We also demonstrate a wide range of crosstalk and competition between SUMO and other major post-translational modifications.

In **Chapter 7**, we describe the PRISM method, which allows for identification of SUMO sites without the requirement for mutant SUMO. Using PRISM, we identify 389 SUMOylated lysines using wild-type SUMO, in over 200 proteins. As such, we take the first step towards the identification of genuinely endogenous SUMOylation sites.

In **Chapter 8**, I summarize and discuss all findings reported in this thesis in a broader context, and provide future perspectives for the field of SUMO proteomics.