# Proteomics and Functional Investigation of SUMO and Ubiquitin E3 ligases

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Cells constitute the tissues of our body and are responsible for producing various changes in response to different situations. For instance, they generate saliva upon seeing food, elevate body temperature to combat pathogens, and initiate the healing process when we suffer wounds. Nonetheless, there are actions that often go unnoticed, such as DNA repair when it becomes damaged. DNA resides within the cell nucleus and can be transcribed and translated into proteins, which play vital roles in numerous cellular processes. While many proteins are naturally present in cells, others are synthesized as needed. However, under cellular stress, such as DNA damage or cell division, instead of producing newly synthesized proteins, the cell relies on modifying existing proteins to carry out essential functions. These modifications can involve the conjugation of small molecules such as ubiquitin (Ub) or Small Ubiquitin-like Modifiers (SUMOs), leading to protein degradation, conformational changes or intracellular relocation of critical proteins. The conjugation cascades of these small molecules involve a well-orchestrated sequence of enzymatic activities performed by dedicated enzymes: E1 (activating), E2 (conjugating), and E3 (ligase). Among these, the E3 ligase enzymes hold significant importance as they confer substrate specificity. These E3 ligases act as molecular "matchmakers," recognizing and binding specific target proteins and facilitating the transfer of the small molecule, such as ubiquitin or SUMO, onto the target.

In this thesis, we have developed an advanced Mass-Spectrometry technology called TULIP2 (Targets for Ubiquitin Ligases Identified by Proteomics 2), which facilitates the identification of ubiquitination targets for specific E3 ligases of interest. Using this technology, we have investigated the BRCA1-BARD1 E3 ligase and explored the in vivo role of the E2 UBE2D3. Furthermore, we have adapted the TULIP2 technology to create the SUMO Activated Target Traps (SATTs), enabling the identification of an E3-specific SUMO proteome.

"In a research project, the hardest part is usually the most exciting one, asking the right questions" Statement 8 of this thesis.

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