

## **Living on the edge: evolutionary adaptation of substrate-recruiting subunits of the cullin-RING ubiquitin ligase complexes to avoid premature degradation**

Maintaining protein homeostasis within a cell is essential for the proper functioning of all living organisms. To prevent the toxic effects of the accumulation of unwanted proteins, cells use sophisticated degradation systems such as the ubiquitin-proteasome system (UPS). The UPS removes target proteins tagged with a small protein, ubiquitin, in a process known as ubiquitination. Ubiquitin is mainly added to a specific site in the target protein - lysine, one of the 20 amino acids that are the building blocks of all proteins. Despite advances in research on how proteins are targeted to UPS, little is known about how functional proteins can avoid it. One intuitive way proteins can escape premature ubiquitination is by getting rid of lysines during evolution. Indeed, in my initial bioinformatics analysis, I found that organisms in the course of evolution increase the fraction of proteins with extensive regions devoid of lysines. Many of these proteins are associated with UPS, suggesting that lysine deprivation may be a hitherto unexplored protein adaptation mechanism.

My main goal is to find an explanation for the widespread occurrence of lysine-deprived regions in proteins. To achieve it, I will combine bioinformatics analyses with experimental approaches. The theoretical part will consist of a quantitative analysis of the evolution of lysine-deficient sequences in multiple taxonomic groups, followed by a qualitative analysis of their biological functions. In the experimental part, I will focus on selected lysine-less proteins that recognize and target substrates for degradation - substrate-recruiting subunits of the cullin-RING ubiquitin ligase complexes. I propose that these recruiters of unwanted proteins *live on the edge* as they are at constant risk of premature ubiquitination, therefore avoiding lysines in their amino acid sequence should be a protective mechanism. To prove this point, I will introduce lysines in regions lacking them using genetic engineering techniques and then monitor their effects on protein stability in human cell lines.

My interdisciplinary research will provide insight into the underlying mechanisms that coordinate the proteolytic network and the expected results will be important as they address central challenges in protein degradation disorders such as neurodegeneration and cancer.