In every cell of the body, proteins are under constant surveillance by a quality-control network that tightly regulates the balance between their biogenesis and degradation. A key component of this network responsible for removing redundant or damaged proteins is the ubiquitin-proteasome system (UPS). The function of molecular chaperones is to promote protein repair. However, if the repair fails, selective degradation is initiated by the small ubiquitin protein (Ub) attachment through ubiquitination. This is mediated by a cascade of enzyme proteins E1, E2 and E3. First, the Ub activated by the E1 enzyme is transferred to the E2 conjugating enzyme, which, with the Ub ligase's participation (E3), transports the Ub to the target protein. E3 ligases are the largest group of enzymes in the UPS system because they play a crucial role in substrate selection. Thus, it is not without reason that E3s enzymes are used for therapeutic purposes due to their ability to regulate the stability of vital cellular components.

The CHIP protein, a member of E3 enzymes, links the chaperone system to the UPS network. To accelerate ubiquitination, CHIP can also cooperate with another E3 ligase, known as UFD-2. However, until now, it has been unclear how the high processivity of this system is achieved. In this project, we investigated the function of the CHIP/UFD-2 complex. Our data show that the CHIP/UFD-2 ligase interaction promotes cooperation between CHIP and E2 enzymes, allowing CHIP to carry out protein substrate ubiquitination much more efficiently. Interestingly, we found that chaperones compete with UFD-2 ligase for binding to the CHIP protein, thus negatively regulating the activity of the complex. Presumably, high CHIP activity is undesirable for chaperones because it may lead to an imbalance between chaperone-mediated protein repair and degradation, inducing the latter. Our studies also show that CHIP activity, triggered by UFD-2, involves the regulation of nonchaperone substrates such as S-Adenosylhomocysteinase (AHCY), an enzyme essential for stimulating metabolic processes in the cell. However, we still do not know the molecular mechanism that controls CHIP activity. Our goal is to understand the molecular mechanisms underlying CHIP regulation by UFD-2 and chaperones using structural biology methods. To this end, I will use cryogenic electron microscopy (Cryo-EM) and X-ray crystallographic studies supported by molecular dynamics simulations. The expected results will be important because they will provide structural information on important components of the UPS system responsible for aging, neurodegenerative diseases, and cancer, among others.