Ubiquitination/SUMOylation Regulate Protein Conformation and Stability via Tag- and Site-Specific interactions

Simran Arora, Debanjana Das, and **Sri Rama Koti Ainavarapu***

Department of Chemical Sciences, Dr. Homi Bhabha Road, Colaba,
Tata Institute of Fundamental Research, Mumbai-400005, India
Email: koti@tifr.res.in
Homepage: https://kotigroup.wordpress.com/

Post-translational modification (PTM) by ubiquitin and ubiquitin-like proteins is a key mechanism for controlling protein fate and function, yet how the modification site influences protein stability and mechanics remains poorly understood. Because substrates often contain multiple lysines in distinct structural contexts, the energetic effects of site-specific ubiquitination can vary widely. Here, we investigated how ubiquitination alters thermodynamic and mechanical stability using the B1 domain of protein L as a structurally well-characterized model. Site-specific conjugation of ubiquitin at seven individual lysines revealed striking positional dependence: modification at K28, located within the α-helix, increased the melting temperature by 12 K and enhanced resistance to mechanical unfolding, whereas other sites had negligible effects. NMR and fluorescence analyses revealed local rearrangements near the α-helix and β3 sheet upon K28 modification, providing a structural basis for the observed stabilization. In contrast, conjugation of the ubiquitin-like protein SUMO1 at the same site produced minimal effects, indicating that the stabilization arises from specific ubiquitin–substrate interactions rather than steric crowding. These results reveal a mechanism by which ubiquitin can function as an allosteric modulator of protein structure and mechanics, illustrating how modification site and tag identity together encode diverse functional outcomes in the proteome.