Chemistry and Biology with the Ubiquitin Signal

Ashraf Brik

Department of Chemistry, Ben-Gurion University of the Negev

In this talk, I will present our novel synthetic approaches for peptide and protein ubiquitination to shed light on the various unknown aspects of the ubiquitin signal in cellular pathways. The attachment of ubiquitin protein to a specific protein target is a widely utilized posttranslational modification in eukaryotes, which is involved in various aspects of cellular functions e.g. protein degradation and DNA repair. Notably, ubiquitination has been implicated in several diseases including cancer and neurodegenerative diseases. In this process, three distinct enzymes, known as the E1-E3 systems, collaborate to achieve a site-specific tagging of the lysine residue(s) in the target protein. The overwhelming majority of studies in the field rely on the in vitro enzymatic reconstitution of this complex posttranslational modification for the protein of interest. However, this process is often challenged by the heterogeneity of the modified protein, the isolation of the specific ligase (E3) and obtaining reasonable quantities of the ubiquitinated protein. Our group reported a highly efficient and site-specific peptide and protein ubiquitination utilizing our developed thiolysine residue and synthetic ubiquitin bearing C-terminal thioester functionality to emulate the action of the enzymatic machinery. This battery of chemical tools allowed for the first semisynthesis of homogeneous ubiquitinated alpha-synuclein to support the ongoing efforts aiming at studying the effect of ubiquitination in health and disease. Additionally, we also achieved the total chemical synthesis of all di-ubiquitin chains as well as the K48-linked tetra-ubiquitin, composed of 304 amino acids. More recently, the synthesis of ubiquitinated peptides linked to mono-, di-, tri-, and tetra-ubiquitin was also made possible, which enabled us to examine the behavior of these novel bioconjugates with several deubiquitinases. We also expanded these approaches to target different deubiquitinases in the ubiquitin system to shed light on their role in health and disease, and ultimately, for drug development.