

**P035** A novel affinity-based purification procedure of human proteasome complexes and proteasome interacting proteins  
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The 26S proteasome is the proteolytic machine of the ubiquitin-proteasome system. This pathway is involved in the degradation of most intracellular proteins and tightly regulates major biological processes. Many studies have demonstrated that a dysregulation of this machinery can lead to various pathologies. The 26S proteasome is a 2.4 MDa complex composed of multisubunit subcomplexes: a core protease, the 20S proteasome, and two regulatory elements, the 19S particles. Despite its physiological importance, many aspects of the mammalian proteasome structural organization and regulation remain to be understood. It is known however that its subunit composition and dynamic association to various proteins regulate its stability and activity. Therefore, we developed a new affinity purification strategy to characterize human proteasome complexes and Proteasome Interacting Proteins. This new single-step procedure, based on the high-affinity binding of a subunit of the human 20S core particle to a monoclonal antibody, allowed the detection of endogenous interactions in erythrocytes without relying on over-expression or tagging strategies. Subsequent proteomic analyses identified all proteasomal subunits, known regulators and recently assigned partners. Moreover, other proteins implicated at different levels of the ubiquitin-proteasome system were also identified for the first time. This novel approach, through the identification of partners affecting proteasomal function, will contribute to a better understanding of this complex proteolytic machine in different physiological cell states.