Large-scale profiling E3-ligases reveals substrate binding specificity determinants

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The ubiquitination is a post-translational signalling event that coordinates not only protein homeostasis yet many different cellular responses such as DNA damage control or the cell cycle. An estimated 600 to 1,000 different E3-ligases orchestrate these signals by identify the correct substrates. However, the specificity determinants of most E3-ligases remain unclear. E3-ligases use different strategies to identify their substrates, such as TRIM21 that uses antibodies, or MDM2 that targets p53 by a short linear motif (SLiM). This study investigates the SLiM-based substrate recognition of over 150 different binding domains of selected E3-ligases using proteomic-peptide-phage display (ProP-PD), enabling for the screen of a million peptides from the human proteome simultaneously for each of the tested domains resulting in the largest data set on potential E3-ligase ligands. Furthermore, we present an array of techniques that validate these interactions through biochemical, bioinformatical and cellular approaches. Out of the 150 different binding domains, 80 have sown to bind to peptides presented by the ProP-PD library. The ProP-PD selections enriched around 8,000 peptides of medium and high confidence of which 281 have been reported before, indicating that 7,336 could represent novel interactions. Further, the binding peptides led to the discovery of 29 new short linear motifs, of which 5 have been validated using fluorescence polarization or peptide arrays. The most interesting examples are the motifs of the PUB domain of RNF31, the SPRY domain of HERC1 and the tandem tudor domain of UHRF1, displaying three different binding interfaces. As well the binding motifs of the MIB domains of MIB1, MIB2, HECTD1, and HECTD1. We further present, validated these interactions in the full-length context using co-immunoprecipitation data.