

**P013** Development of TUBEs (Tandem Ubiquitin Binding Entities) to study ubiquitin dependent processes

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Ubiquitylation is the process where a lysine in a substrate protein is covalently bonded to ubiquitin. Modification of the substrate may be in the form of mono-ubiquitylation or poly-ubiquitylation. Ubiquitin binding domains (UBDs) is a diverse family of structurally dissimilar protein modules which bind mono- and poly-ubiquitin. One of the best described UBDs is the Ubiquitin Associated (UBA) domain. Proteasomal degradation and de-modification of substrates by de-ubiquitylating enzymes (DUBs) result in technical obstacles when attempting to analyze the ubiquitylation status of a protein. Although proteasomal inhibition can inhibit proteolytical activities in the proteasome and allow analysis of modified protein, this has been shown to also have effects on protein synthesis. In order to avoid DUB activity and preserve modified substrates, it is necessary to use general cysteine protease inhibitors, such as iodoacetamid or N-Ethylmaleimide (NEM). The observation that more than one UBA domain can be found in a single protein led us to speculate that repeated UBA domains may bind poly-ubiquitin cooperatively. Here, we have developed Tandem Ubiquitin Binding Entities (TUBEs), based on model UBA domains. Engineered multiple UBA domains were developed as GST fusion proteins. These exhibited increased affinity for poly-ubiquitin, and poly-ubiquitylated proteins *in vitro*. Experiments were done using either GST-TUBEs coupled to glutathione agarose, or BiaCore affinity measurements.