Protein purification

The proteins that were purified for utilization in \textit{in vitro} ubiquitination assays were overexpressed in \textit{E. coli} strain BL21(DE3) and isolated from the soluble fraction of cleared cell lystates that were prepared by sonication as previously described (Cyr et al., 1992). Hsc70 was purified by a combination of ATP-agarose and anion exchange chromatography (Cyr et al., 1992). Hdj-2 was purified by anion exchange and hydroxyapatite chromatography (Meacham et al., 1999). UbcH5a, Ubc5a C85A, Ubc7, Ubc6 1-243, CHIP, CHIP K30A, CHIP H260A, and CHIP P269A were His\textsubscript{6} tagged and purified by metal chelate chromatography (Lu and Cyr, 1998). Proteins in peak fractions were greater than 90\% pure, concentrated to 1-2 mg/ml, dialyzed in a buffer that contained 20 mM Hepes, pH 7.4, 150 mM NaCl, 1 mM \(\beta\)-mercaptoethanol and 0.1 mM phenyl methyl sulfonylfluoride, and then snap frozen and stored at \(-80^\circ\text{C}\).

Gst-NBD1-R was expressed from pGEX-5X-NBD1-R in \textit{E. coli} strain BL21 (DE3) and purified as follows. Cells from a 600 ml culture were harvested after a 16-hour induction period at 30 \(^\circ\text{C}\) with 0.2 mM IPTG. Cell pellets were resuspended and incubated for 30 min. on ice in a buffer composed of 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 150 mM NaCl, 1mM phenylmethyl sulfonyl fluoride, 1mM DTT, and lysozyme 0.1ug/ml. The cell suspension was then supplemented with 1\% sarkosyl and the slurry was sonicated. The cell extract was cleared of insoluble debris by centrifugation and the supernatant was supplemented with glutathione-agarose beads and incubated at 4 \(^\circ\text{C}\) for 1 hr. The glutathione-agarose beads were pelleted and washed 3 times with ice-cold phosphate buffered saline (PBS). The bound gst-NBD1-R was eluted with 50 mM Tris-HCl that contained 10 mM reduced glutathione. Gst-NBD1-R was typically eluted at a concentration near 2 mg/ml and was flash frozen and stored at \(-80^\circ\text{C}\).