Designing a molecular probe for detection of mixed-linkage polyubiquitin chains

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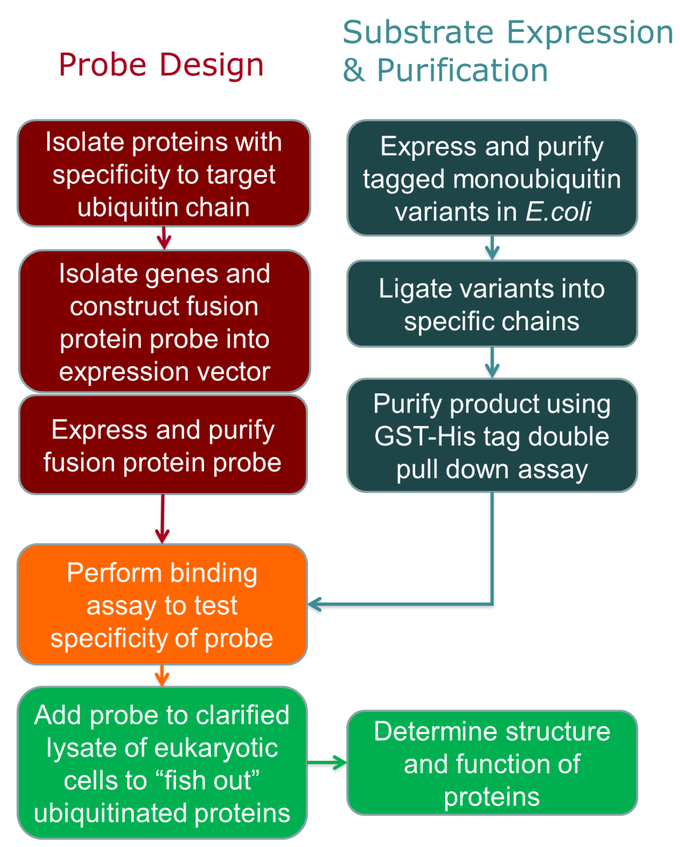
Introduction

Ubiquitin is a small protein that is key in several regulatory process in cells, and is found in nearly all eukaryotic organisms. The addition of ubiquitin to a substrate protein is called ubiquitination. Ubiquitination can affect proteins in many ways: it can signal for their degradation via the proteasome, alter their cellular location, affect their activity, and promote or prevent protein interactions. The structure of these ubiquitin chains are incredibly diverse, however their functions are poorly understood. The goal of this experiment is to design a molecular probe that is able to specifically recognize different types of ubiquitin chains, and characterize the proteins that they are targeting to better understand the purpose of specific ubiquitin chain forms.

Keywords: Ubiquitin, protein degradation, probe

Procedure



Results

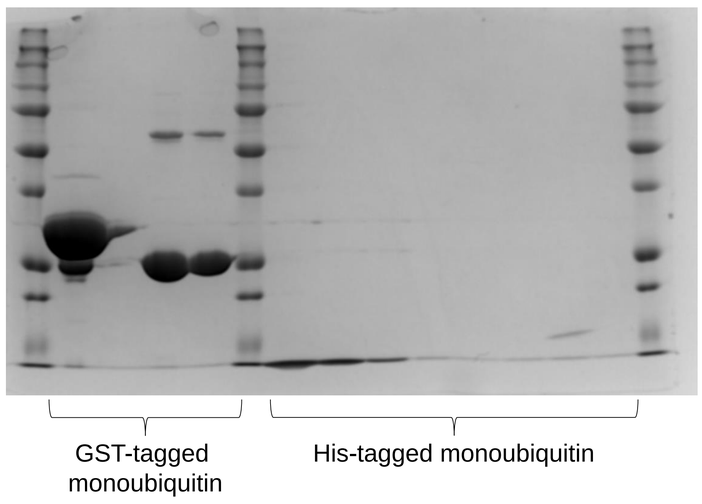


Figure 3 | SDS-PAGE gel electrophoresis of purified monoubiquitin subunits for synthesis of polyubiquitin chain. GST-tagged monoubiquitins ~34kD, His-tagged monoubiquitin ~8kD.

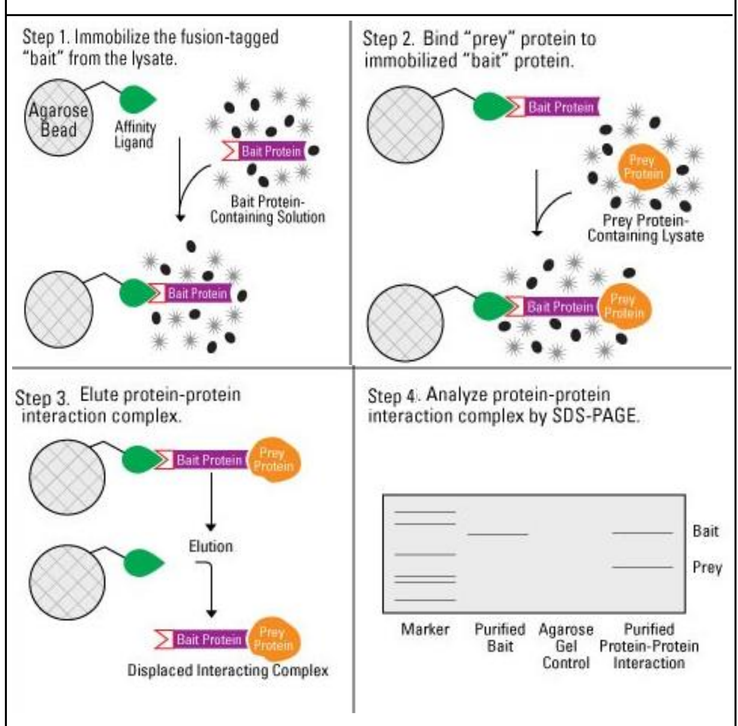


Figure 4 | Pull Down Assay for tagged monoubiquitin subunits. A) Glutathione agarose beads bind to GST-tagged "bait"protein. B) GST-tagged "bait" protein interacts with His-tagged "prey" protein. C) GST-His complex is eluted off glutathione beads. D) Results analyzed using SDS-PAGE will show presence of ligated polyubiquitin chain in elution. Thermo Fisher. "Pull-Down Assays." Thermo Fisher Scientific. 2004.

Research

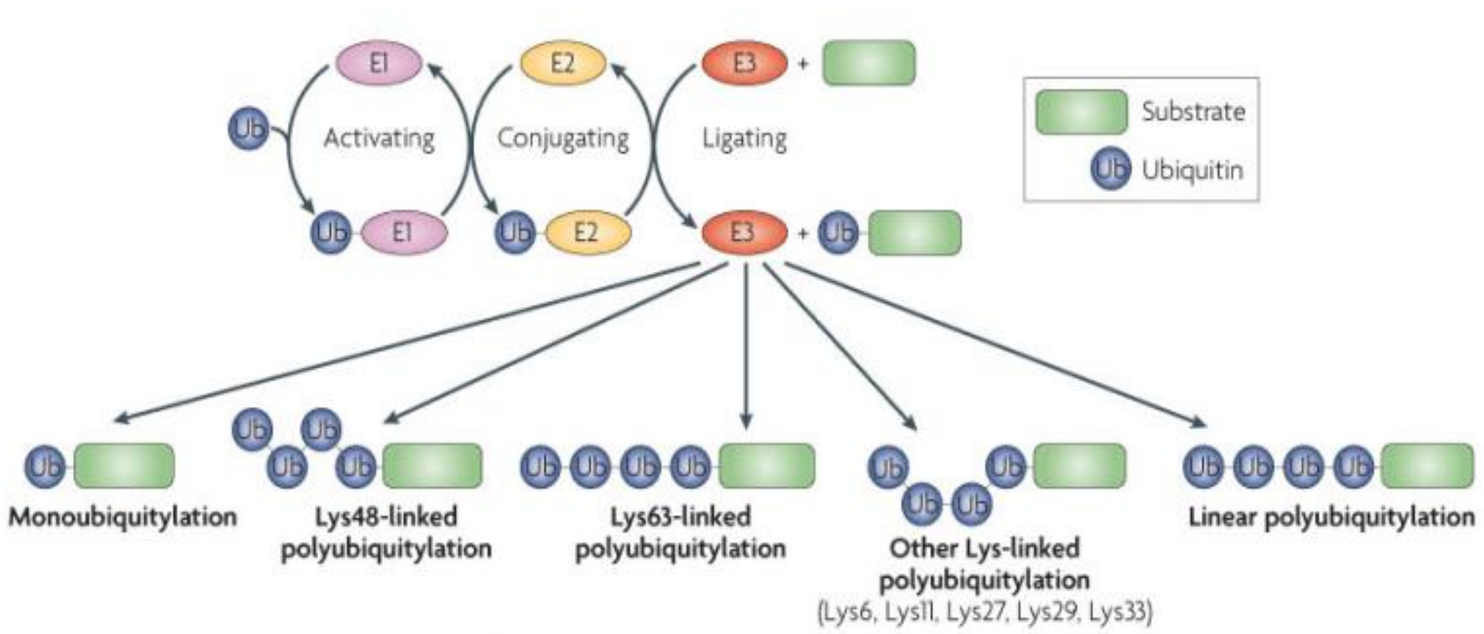


Figure 1 | **Enzymatic cascade that leads to substrate ubiquitylation.** The activity of three enzymes is required for ubiquitylation: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin-ligating enzyme (E3), which recognizes the substrate. The completion of one ubiquitylation cycle results in a monoubiquitylated substrate. However, the cycle can be repeated to form polyubiquitylated substrates. Additional ubiquitin molecules can be ligated to a Lys residue (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 or Lys63) in a previously attached ubiquitin to form Lys-linked chains.

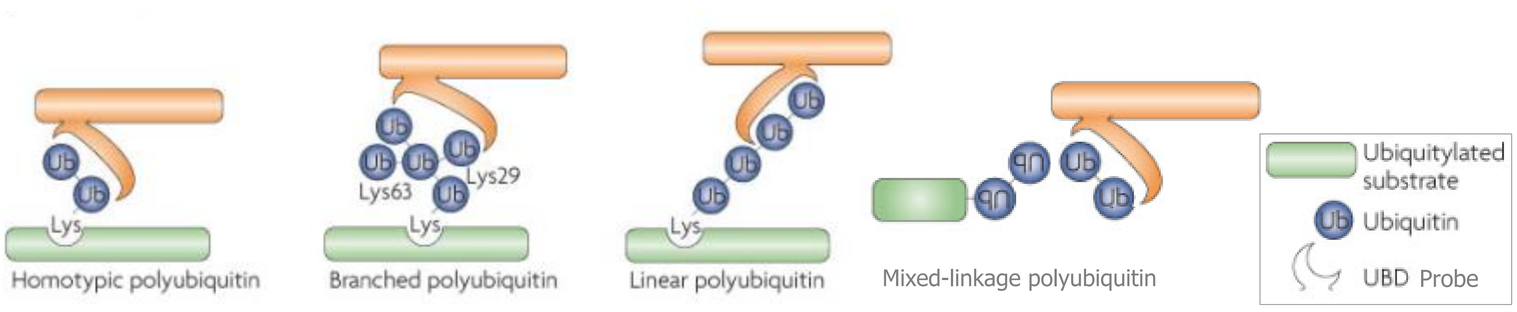


Figure 2 | **Detection of polubiquitin chains by fusion probe.** Probes will be specifically designed to interact with different kinds of ubiquitin chains. Branched, linear and mixed linkage polyubiquitin chains will interact with specific ubiquitin binding domains of fusion probe.

Dikic, Ivan, Soichi Wakatsuki, and Kylie J. Walters. "Ubiquitin-binding Domains — from Structures to Functions." *Nature Reviews Molecular Cell Biology Nat Rev Mol Cell Biol* 10.10 (2009): 659-71.

Conclusions and Future Work

Protein production has been successful, and next steps involve creating and purifying probes. Further testing and binding assays will be necessary to unsure specificity of probe.

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