# Activity of USP7 Influenced by the Nature of POLI Interaction with TRAF and UBL1-2 Sites

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## Introduction

Ubiquitination is a diverse cellular process that occurs in various different organisms, including but not limited to: humans, animals, and yeast (Laney, 1999). It generally aims to degrade structurally inadequate proteins that have been misfolded or damaged (Laney 1999). It has been discovered that in this system, a small ubiquitin molecule covalently bonds to its substrate and tags it for degradation (Laney 1999). Deubiquitination is a negative regulator of intracellular protein degradation, and it is performed by deubiquitinating molecules. The binding of ubiquitin to a deubiquitinating enzyme (DUB) forces the DUB to fold into an active conformation and cleave the targeting ubiquitin molecule (Amerik, 2004). This cleave occurs after the 76th residue of ubiquitin, effectively detaching it from the molecules it marks for degradation by proteasomes (Peters, 2013). USPs are considered the largest family of DUBs, and one specific and versatile USP happens to be USP7 (Reyes-Turcu, 2009). This molecule has two major interacting sites which are localized to the TRAF domain, located within the N-terminal of the enzyme, and the UBL1-2 domain, located within the c-terminal tail of USP7 (Hu, 2006)(Rougé, 2016). The binding of substrates to both domains largely affects USP7 activity (Holowaty, 2003). The UBL1-2 sites interact with (R/K)xKxxxk motifs; segments of protein substrates with this structure of residues have the ability to interact with this site on USP7 (Pfoh, 2015). It has also been discovered that the TRAF domain of USP7 recognizes P/A/ExxS motifs (Hu, 2006). It has recently been discovered that a protein substrate called polymerase iota (POLI) interacts with both of the sites on USP7 enzyme (Bezsonova, 2021). The TRAF interaction between the molecules has been mapped between the 421st and 590th residues of polymerase iota (Bezsonova, 2021). This is 1 of the 18 P/A/ExxS motifs present on POLI (Bezsonova, 2021). Additionally, the interaction of POLI with the UBL1-2 domain has been analyzed mainly between the 438th and 448th residues of POLI (Bezsonova 2021). Despite these discoveries, it is still unclear how exactly the binding of POLI to one, both, or none of the USP7 sites influences the latter's activity and deubiquitination of tagged proteins. It's important to study this, for the ubiquitin system is widely involved in the cell cycle, transcription, signal

transduction, protein sorting, neuropathology, cancer, virology, and immunology (Peters, 2013). USP7 specifically has been seen to be deregulated in cancers and neurodevelopmental disorders (Fountain 2019). This study aims to examine how POLI's characteristic binding at USP7's TRAF and UBL1-2 domains affects its conformation and subsequent ability to cleave ubiquitin-tagged molecules.

## Proposed Methods

1) We will be designing three mutants of POLI
    a. Mutation on the TRAF binding motif (S580A)
    b. Mutation on the UBL1-2 binding motif (K440A, K444A, K446A)
    c. Mutation on both the USP7 binding motifs

2) The methods used to answer the research question would be
    a. Site directed mutagenesis and
    b. Enzyme kinetics (interaction of Pol i mutats with USP7)

USP7 alone and in complex with the mutants will be mixed with the fluorogenic substrate ubiquitin 7-amido-4-methylcoumarin (Ub-AMC). We will then measure the release of AMC which will be detected through the enzymatic assay. Higher versus lower levels of released AMC will enable us to determine the change in activity of USP7.

**We will be using recombinant DNA, but no approval was needed for this experiment.**

## Research Team

I am working alongside Dr. Bezsonova, Dr. Jaiswal, and Dr. Semenova on this project. Dr. Bezsonova is the principal investigator. Dr. Jaiswal is the postdoctoral fellow who was engaged in the performance and design of the lab experiments. Dr. Semenova assisted with the logistics, and she helped design primers that would yield recombinant DNA. I was responsible for assisting Dr. Jaiswal with the experiments (mostly the PCR reactions), running the enzymatic assays, and interpreting/synthesizing the results.

## References


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