Investigating the effects of phosphorylation on the BRCA1/PALB2 interaction

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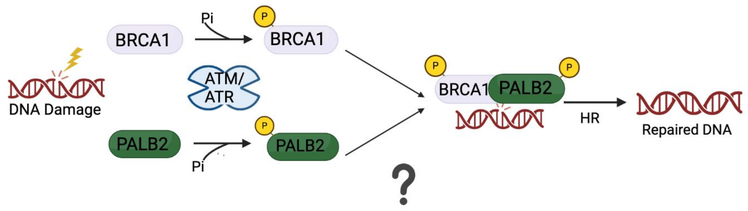
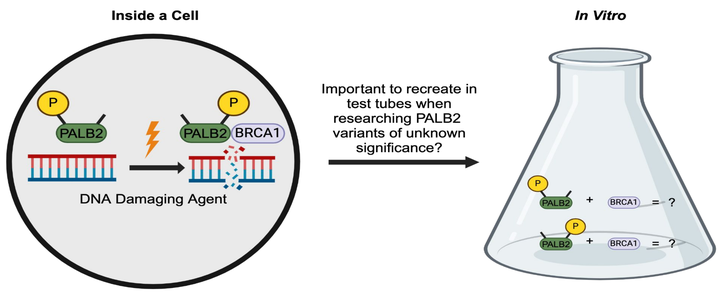
Introduction

Does phosphorylation promote efficient BRCA1/PALB2 binding?

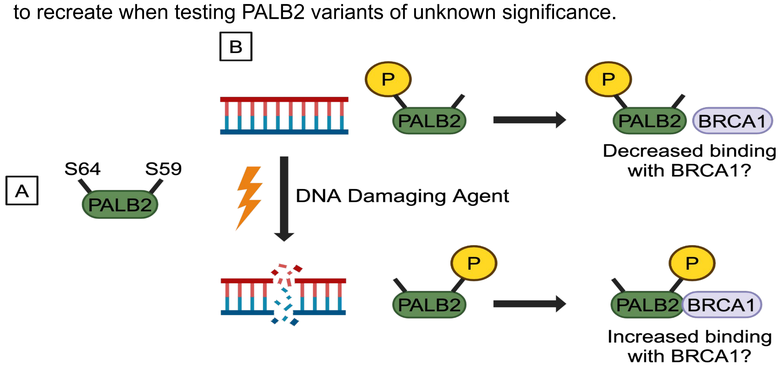


Above: In the presence of DNA damage ATM/ATR is activated, resulting in the phosphorylation of BRCA1 and PALB2. Upon phosphorylation, BRCA1 and PALB2 form a heterodimer resulting in repaired DNA through homologous recombination.

Does phosphorylation affect the binding interaction *in vitro*?



Above: In cells, PALB2 is phosphorylated upon detection of DNA damage, which affect the BRCA1-PALB2 binding interaction. However, it is unknown if phosphorylation affects the binding interaction *in vitro* and thus would be important to recreate when testing PALB2 variants of unknown significance.



Above: (A) PALB2 residues of interest (S59, S64) that are phosphorylated upon DNA damage. (B) In the absence of DNA damage, S64 is phosphorylated. In the presence of DNA damage, S64 is hypophosphorylated, and S59 is phosphorylated.

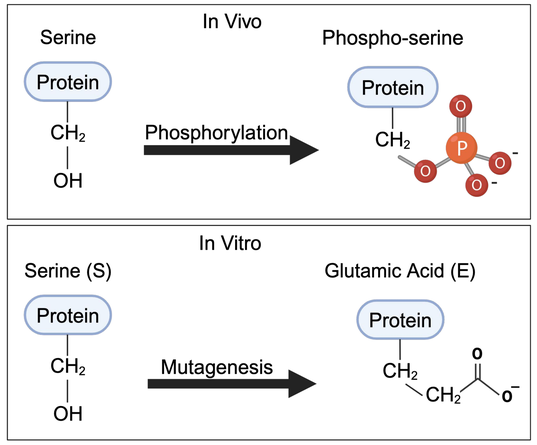
Objective

Recreate phosphorylation *in vitro* via a phosphomimicking mutant to determine its effect on the binding interaction between BRCA1 and PALB2.

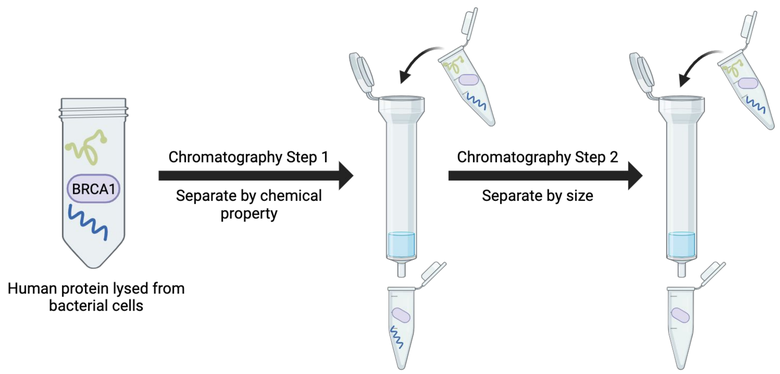
Methods

Site-Directed Mutagenesis

Right: *In vivo* phosphorylation involves the attachment of a phosphate group to serine residues. To mimic this state *in vitro*, site-directed mutagenesis is used to mutate a serine (S) to a glutamic acid (E), providing a negative charge that mimics phosphorylation.

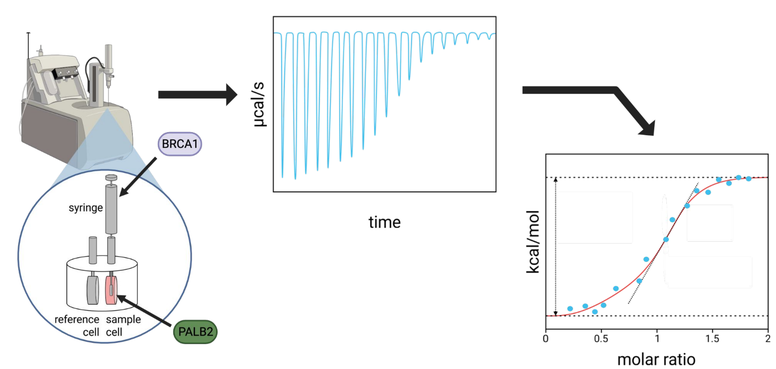


Protein Purification



Above: After transformation and expression of human protein in bacterial cells, protein is lysed from bacterial cells and purified via two methods of chromatography. The first step involves purifying the protein utilizing a chemical property specific to the human protein. The second step utilizes size to further purify the protein of interest.

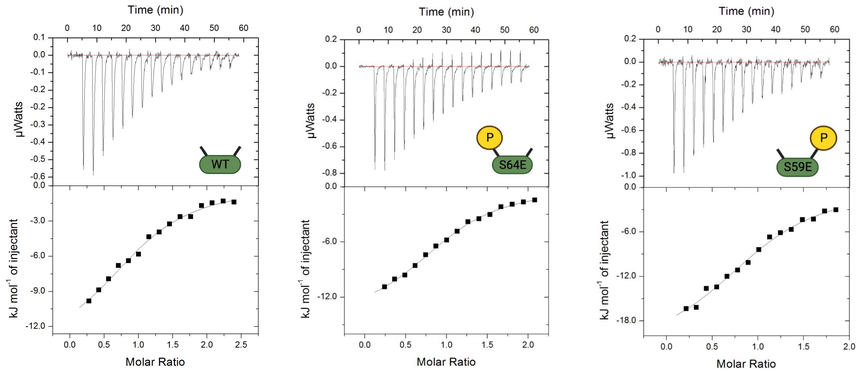
Isothermal Titration Calorimetry



Above: Schematic describing the steps of isothermal titration Calorimetry

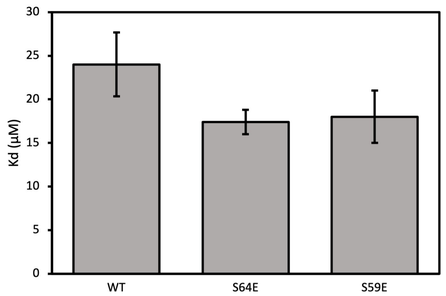
Results

Isothermal Titration Calorimetry



Above: Isothermal Titration Calorimetry experiments showing the titration of Wild Type (WT) BRCA1 into WT, S64E, and S59E PALB2.

BRCA1-PALB2 Binding Interaction Kd



Above: Kd of PALB2 WT, S59E, and S64E binding interaction with BRCA1 WT

Conclusions and Future Directions

- No biologically significant binding differences between PALB2 WT, PALB2 S64E, or PALB2 S59E *in vitro*
- PALB2 phosphorylation is not important to recreate when testing the effects of PALB2 variants of unknown significance (VUSs) on the BRCA1-PALB2 binding interaction *in vitro*
- Further research is needed to determine the effect of BRCA1 phosphorylation on the BRCA1-PALB2 binding interaction *in vitro*

References and Funding

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