Visualizing the Structural Effects of Proline Variants on the BRCA1-PALB2 Binding Interface

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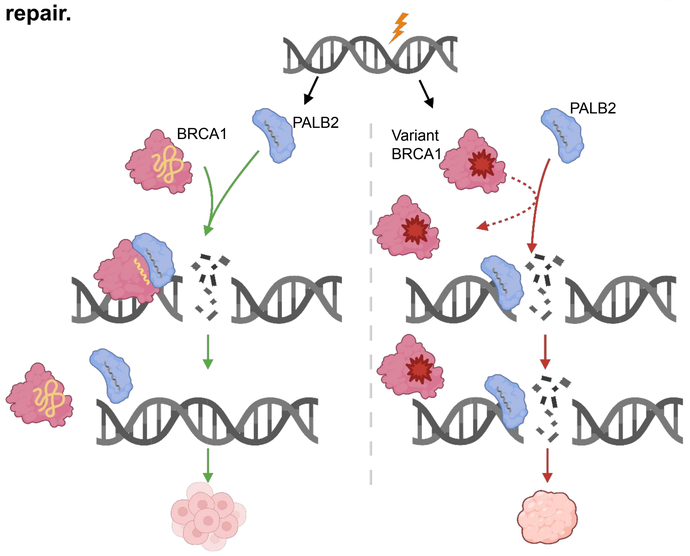
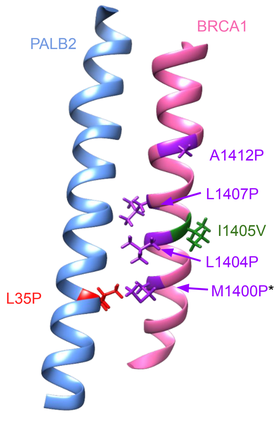
Introduction

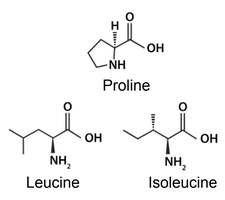
BRCA1 and PALB2 interaction is essential for DNA damage repair.



Above left: Interaction between wild-type BRCA1 and PALB2 repairs DNA damage and leads to the proliferation of healthy cells. **Above right:** BRCA1 variants may disrupt interaction with PALB2 and inhibit DNA repair, leading to tumorigenesis.

Proline variants in BRCA1 may disrupt alpha-helix formation and inhibit interaction with PALB2.



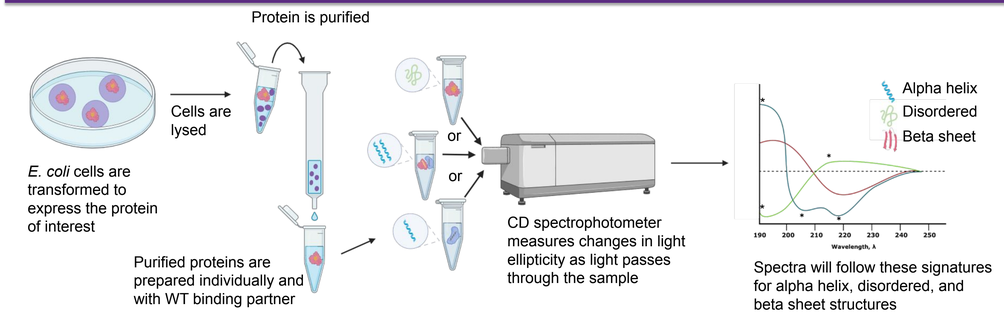


Above: Comparison of proline, leucine, and isoleucine. The secondary amine in proline can disrupt protein structure.
Left: BRCA1 and PALB2 cartoon structure with location of variants. PDB ID: 7K3S
Variants of unknown significance.
Likely benign variant.
Pathogenic variant.
*Variant not yet found in patients.

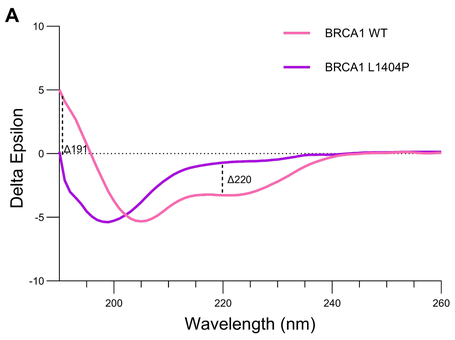
Objectives

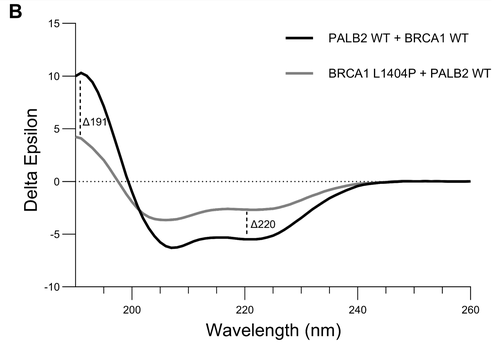
- Express and purify shortened constructs of BRCA1 and PALB2 variants
- Visualize the structure of wild type (WT) BRCA1 and WT PALB2 individually and together using circular dichroism (CD)
- Repeat the above step with each variant construct and its WT binding partner

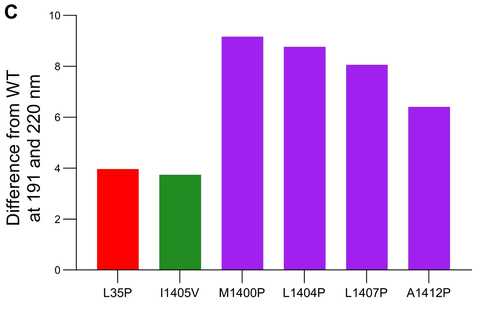
Protein purification and circular dichroism

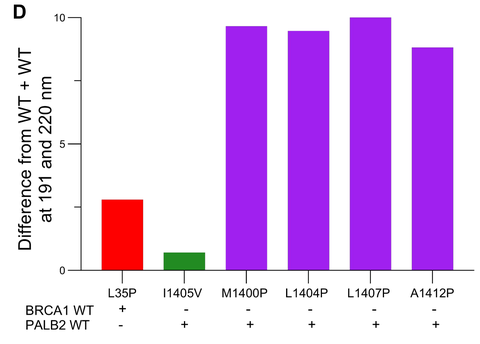


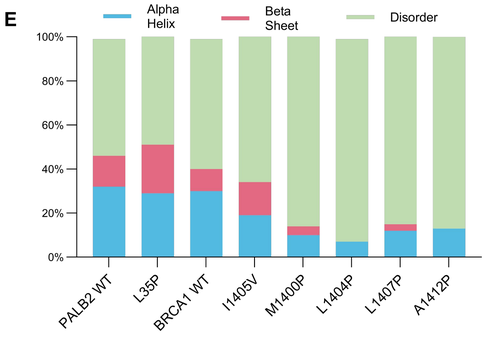
Proline variants disrupt BRCA1 structure











A. CD spectra of BRCA1 WT and BRCA1 L1404P. Δ191 and Δ220 are highlighted since they will be proxies to measure the differences between spectra as depicted in C, D.

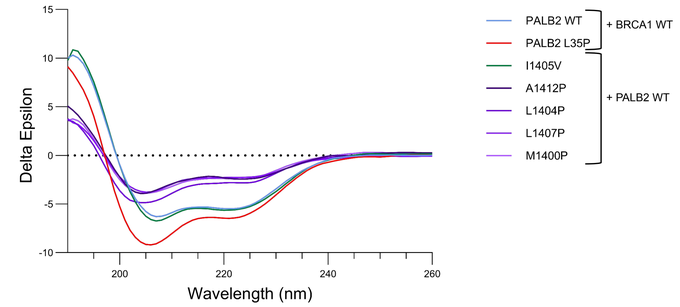
B. CD spectra of BRCA1 WT + PALB2 WT and BRCA1 L1404P + PALB2 WT.

C. Each construct compared to its WT counterpart. L35P is compared to PALB2 WT while the other variants are compared to BRCA1 WT. Total difference = |Δ191| + |Δ220| , as shown in A, B.

D. Each construct in combination with its WT binding partner compared to the WT + WT spectra. Difference is measured using the same method described in C.

E. Deconvolution from DichroIDP estimates the percent of each secondary structure present.

Conclusions and future directions



Protein	Variant	Located in binding pocket?	Found in patients?	Difference Compared to BRCA1 or PALB2 WT	Difference Compared to WT-WT
BRCA1	I1405V	×	✓	↑↑	↑
	A1412P	×	✓	↑↑↑	↑↑↑
	L1404P	✓	✓	↑↑↑	↑↑↑
	L1407P	✓	✓	↑↑↑	↑↑↑
	M1400P	✓	×	↑↑↑	↑↑↑
PALB2	L35P	✓	✓	↑↑	↑↑

- **BRCA1 demonstrates increased helicity upon binding to PALB2**
- **BRCA1 variants compared to BRCA1 WT:** All variants increased the disorder of BRCA1 WT.
- **BRCA1 variants in combination with PALB2 WT:** I1405V had nearly identical structure to BRCA1 WT in the presence of PALB2. Each BRCA1 proline variant demonstrated similar increases in disorder compared to the WT + WT spectra.
- In combination with binding and functional data, proline variants that disrupt the structure of the BRCA1-PALB2 heterodimer may be pathogenic
- **Future studies:** Correlate structural findings with homologous recombination assays to determine the relationship of structural effects with pathogenicity. Determine structural changes on the amino acid level.

References and Funding

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Thank you to TCU College of Science and Engineering and NIH for funding.