A Kink in the System: The Consequences of Proline Mutations in PALB2

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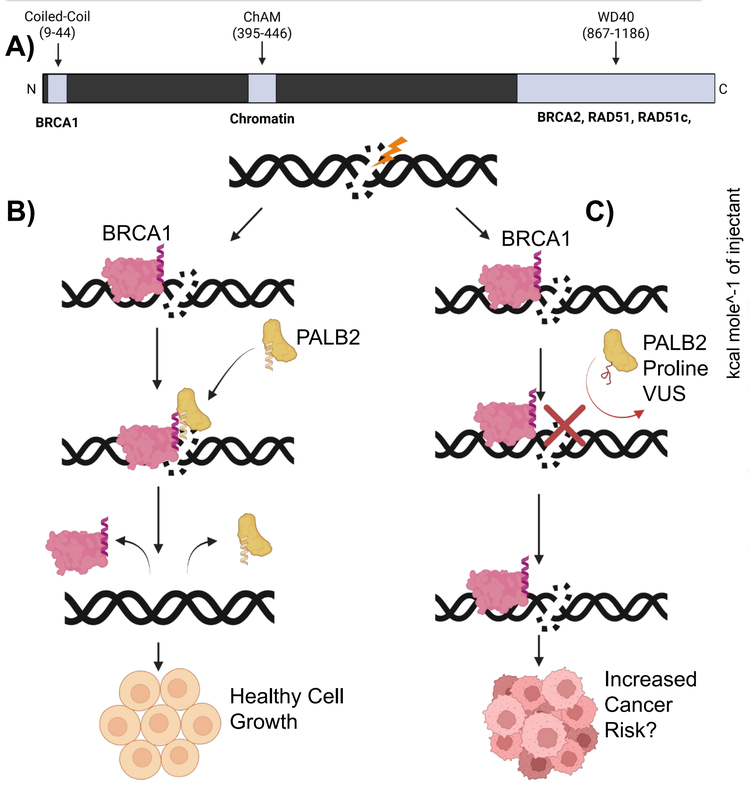
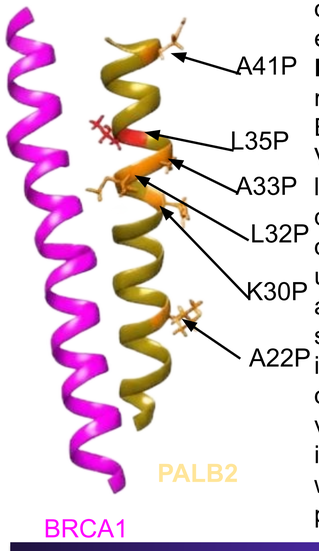
PALB2 is a vital component in efficient DNA Repair



A) The protein length of PALB2 with corresponding binding partners. **B)** Appropriate heterodimerization of BRCA1 and PALB2 lead to efficient DNA damage repair. **C)** Proline VUS may perturb the coiled-coil structure and disrupt binding with BRCA1 potentially increasing cancer risk

Proline variants in PALB2 may abrogate both structure and function

Variant	Sequence alignment: Residues 1-44	Category
WT	MDEPPGKPLSCEEKEKLKEKLAFLKREYSKTLARLQRAQRAEKI	Reference
A22P	MDEPPGKPLSCEEKEKLKEKL**P**FLKREYSKTLARLQRAQRAEKI	Backside
K30P	MDEPPGKPLSCEEKEKLKEKLAFLKREYS**P**TLARLQRAQRAEKI	Backside
L32P	MDEPPGKPLSCEEKEKLKEKLAFLKREYSKT**P**ARLQRAQRAEKI	Interface
A33P	MDEPPGKPLSCEEKEKLKEKLAFLKREYSKTL**P**RLQRAQRAEKI	Backside
A41P	MDEPPGKPLSCEEKEKLKEKLAFLKREYSKTLARLQRAQR**P**EKI	Backside

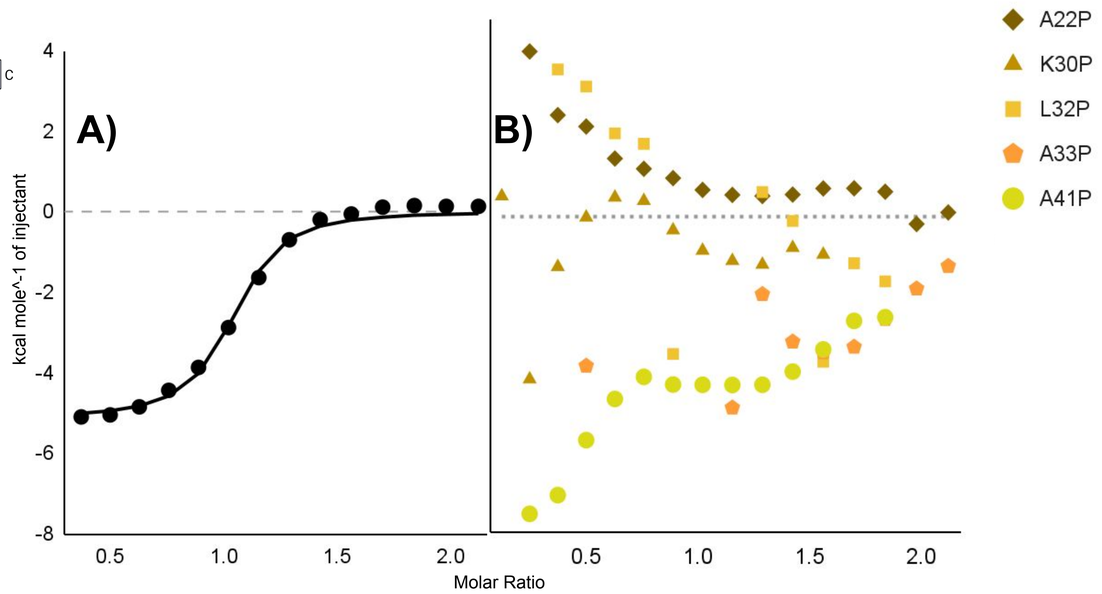


Above: The aligned sequences of WT PALB2 with each VUS with each proline substitution shown **Left:** Structure of the coiled coil regions of both PALB2 and BRCA1 with positions of PALB2 VUS labeled. L35P has been linked in multiple studies to the development of breast and ovarian cancer. However, it is unknown if it is due to an amino acid substitution or destruction of secondary structure due to the introduction of a proline. The other five VUS are also proline variants; in this study we have investigated their binding ability with BRCA1 and plan to further probe their secondary structure.

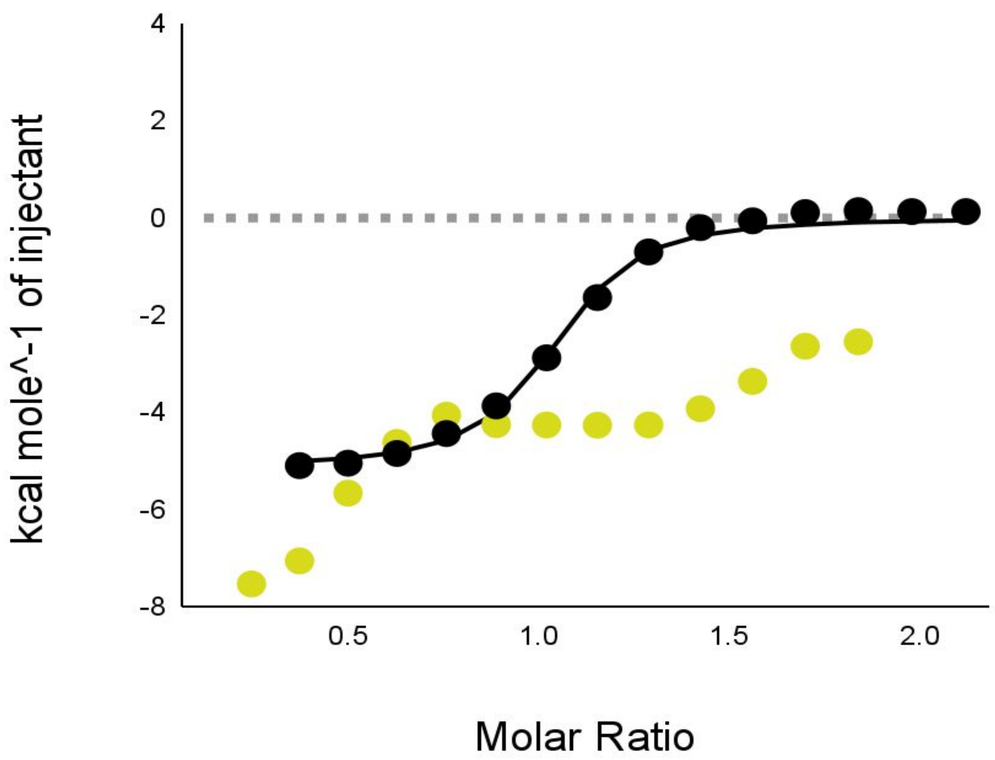
Objectives

I. Characterize the extent of alpha-helical disruption induced by site specific proline substitutions
II. Evaluate the necessity of a rigid coiled-coil architecture for the high-affinity recruitment of BRCA1 to the PALB2 N-terminus.
III. Assess the potential pathogenicity of unclassified *PALB2* variants by correlating structural collapse with clinical cancer risk profiles.

Proline substitutions illustrate alterations/disturbance in binding pattern with BRCA1

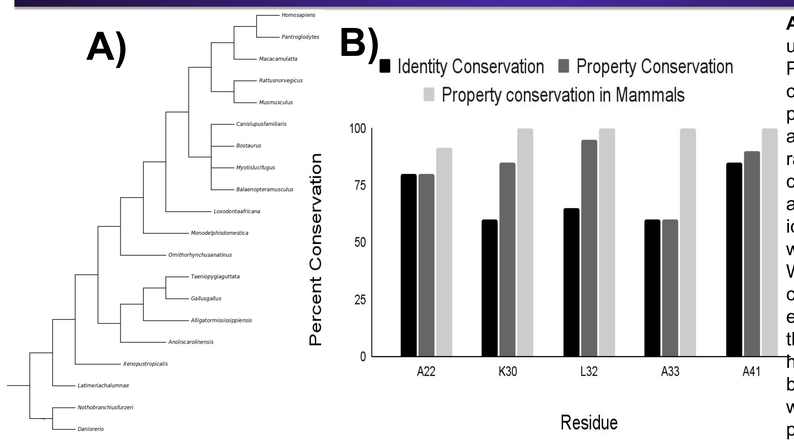


C) K_d= 0.578 µM ± 0.128 µM



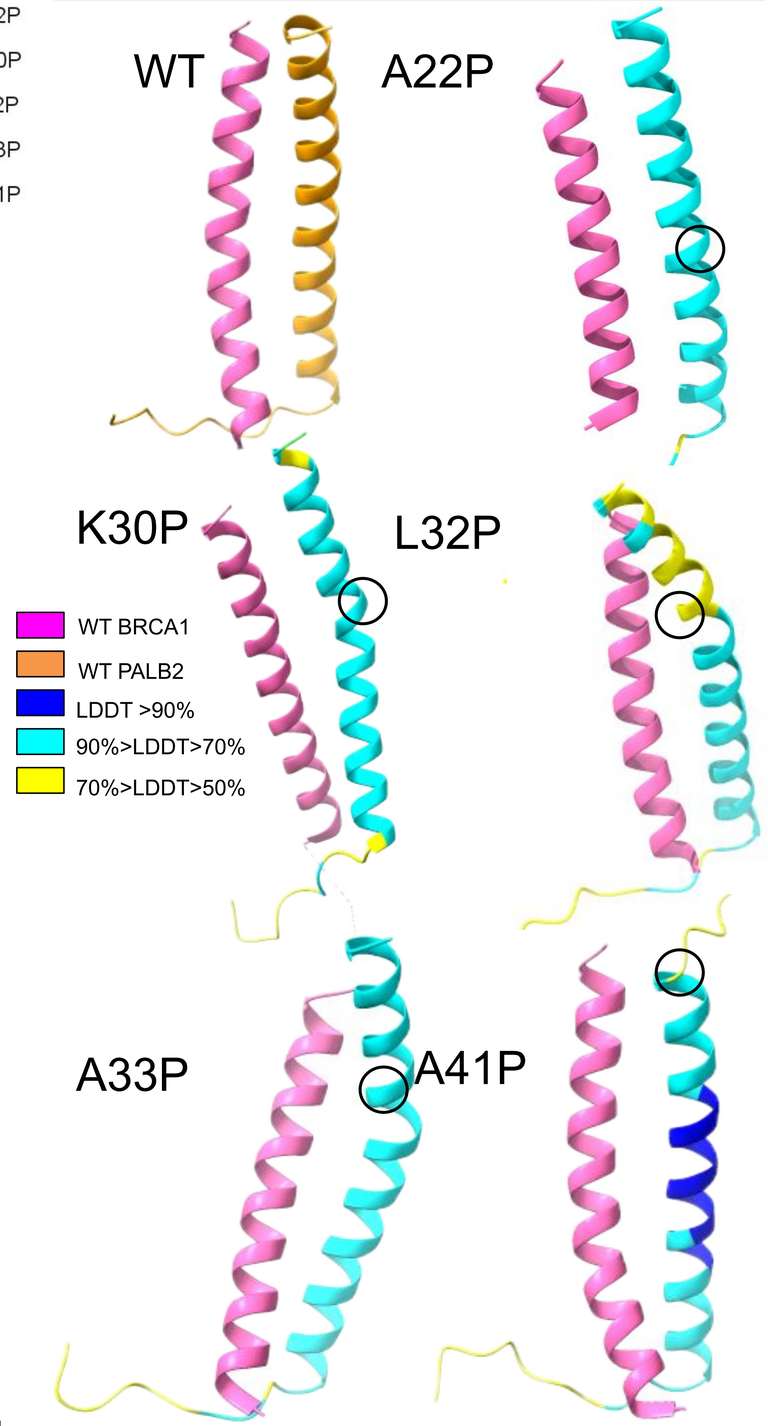
A) Binding isotherm of WT PALB2 titrated with BRCA1. The gradual heat release and resulting sigmoidal curve are indicative of a binding event and create the baseline negative control for our experiment. **B)** Comparative ITC profile of proline substitution PALB2 variants. VUS K30P, L32P and A33P show that binding has been completely disturbed. Interestingly VUS A22P and A41P show a non normal binding interaction that may need to be investigated further to determine if function is completely terminated. **C)** Calculated K_d of the binding interaction between WT PALB2 and BRCA1. **D)** Enlarged non-normal binding profile of A41P overlaid with WT PALB2 binding data and fitted line, contrasting the differences in binding with BRCA1. Surprisingly the A41P variant displayed a more exothermic initial heat of binding. This finding could convey a few possibilities, like increased hydrophobic packing, an enthalpy-entropy compensation or the facilitation of novel interactions in the interface as the coil, may coil more tightly.

Residue conservation within the coiled-coil domain of PALB2



A) Phylogeny tree of vertebrates used in the alignment of the PALB2 coiled-coil domain consisting of 12 mammals (3 primates), 2 birds, 2 reptiles, 1 amphibian, 1 lobed fish, and 2 ray finned fish. **B)** Ratio of conservation of amino acids across species, evaluating the identity, property, and property within mammals conservation. We see that property is majorly conserved across species especially in mammals marking these sites as evolutionarily highly important and would likely be sensitive to mutation that would disrupt the original property.

Structure prediction and future assessment



Above: The predicted structure of the PALB2-BRCA1 heterodimer, as predicted through Alpha Fold (Google Deepmind). Pink color corresponds with BRCA1 and orange being WT PALB2. The dark blue color corresponds with a high LDDT score (>90), light blue a moderate LDDT score (<90>70) and yellow a lesser LDDT score (<70>50). These values are how confident the algorithm is that PALB2 is in these exact positions. Interestingly the coiled-coil is not completely broken in any of these but noticeable kinks are formed and A41P has an area of high confidence still participating in the coiled-coil structure and binding with BRCA1. Because these models are not entirely confident we intend on using CD to probe the structure further

References and Funding

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