What's Linker Have to Do With It?
Examining the Structure & Stability of Palladin's Ig3-4 Linker Region

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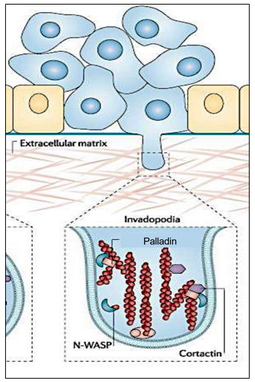
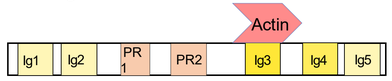
Introduction

Actin
- The most abundant protein within eukaryotic cells
- Plays a role in cell motility, muscle contraction,cell shape, cytoskeleton support, and cell division

Palladin
- Involved in embryonic development and cancer metastasis
- Made up of 5 immunoglobulin (Ig) domains
- Ig3 is the minimum domain required for actin binding
- Ig3-4 has no actin binding affinity, but it increases the binding ability when paired with Ig3

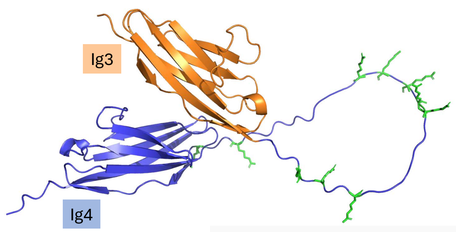


Nürnberg, A., Kitzing, T. & Grosse, R. *Nat Rev Cancer* 11, 177–187 (2011). https://doi.org/10.1038/nrc3003



Hypothesis

Ig 3-4 Linker Region
- Consists of 41 amino acids; most Ig domain linkers are significantly shorter
- Predicted to be intrinsically disordered



Ig3-4 Linker Mutations
10 arginines within the Ig3-4 linker region were mutated to alanines, creating **RLinkerA**

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34LinkerWT    AVNQRGRSPRSPSGHPHARRPRSRSRDSGDENEPIQERFFR
QELinkerA     AVNARGRSPRSPSGHPHARRPRSRSRDSGDANAPIAARFFR
RLinkerA      AVNQAGASPASPSGHPHAAAPASASADSGDENEPIQEAFFA
QELinkerR     AVNRRGRSPRSPSGHPHARRPRSRSRDSGDRNRPIRRRFFR
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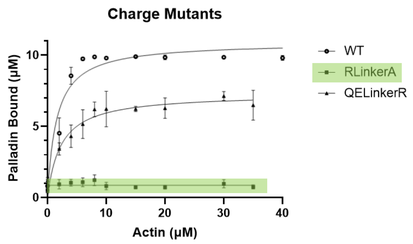


Charge neutralization (**RlinkerA**) abolishes actin-binding ability

Increasing positive charge has the least amount of change on the K_d value (QELinkerR K_d=2.3 mM), but it has a much lower B_{max} than wild-type palladin

Methods & Procedures

Purification
- Thawed, sonicated, and centrifuged pellets of wild-type and mutant palladin

Ni-NTA Column
- Isolated and purified the His-tagged proteins

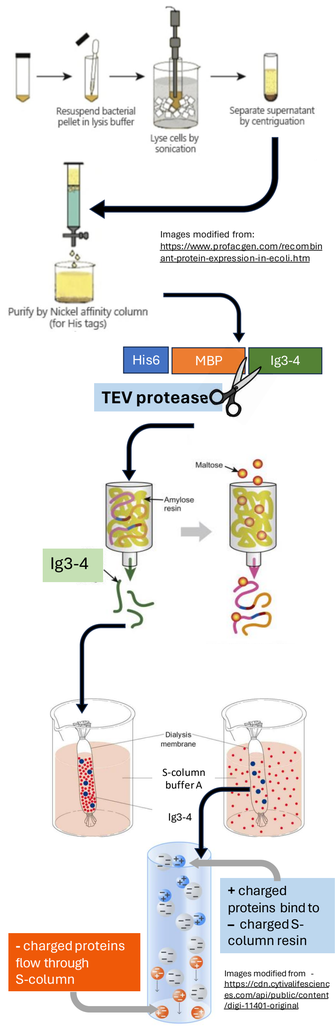
Adding TEV-Protease
- Cut the MBP-His tag from the palladin proteins

Amylose Column
- Isolated the Maltose binding protein (MBP), a solubility tag, from the palladin protein

Dialysis
- Placed the palladin protein samples in Buffer A solution overnight
- Buffer A is composed of 25 mM KH_2PO_4, 25 mM NaCl, 2 mM DTT, pH 5.5

S-column (Cation Exchange – SP Sepharose)
- Eluted palladin from S-column with 1 M NaCl gradient to obtain pure protein

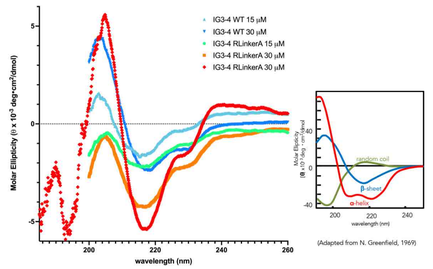
Circular Dichroism (CD) Spectroscopy
- Protein samples were loaded into a cuvette and subjected to beams of polarized light
- Collected absorption data between 260 nm – 200 nm
- Subjected samples to thermal denaturation conditions of 20°C to 90°C



Images from: Pignataro, María & Herrera, María & Dodero, Veronica. (2020). Evaluationof Peptide/Protein Self-Assembly and Aggregation by Spectroscopic Methods. *Molecules*. 25: 4854.

Results

Circular Dichroism



CD wavelength scan of WT and RlinkerA mutant of Ig3-4 at two different protein concentrations shown at left with standard spectra for different protein secondary structures shown at right.

DichroWeb Results
- Secondary structure was analyzed from CD scan

Ig3-4 Wildtype Linker Region

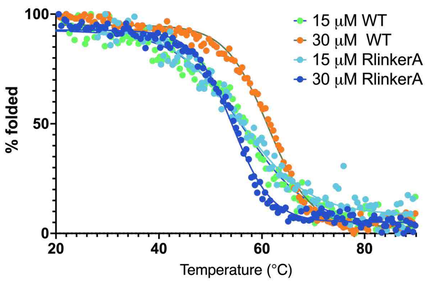
Alpha Helix	Beta Sheet	Random Coil
4%	49%	47%

Ig3-4 R Linker A Mutant Region

Alpha Helix	Beta Sheet	Random Coil
9%	79%	13%

Maximum Error for all Scans: **Less than 0.22**

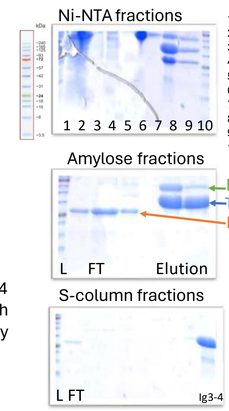
Thermal Denaturation of Ig3-4



Ig3-4	T_M (°C)	Hill Slope
15 μM WT	56.31	-0.067
30 μM WT	61.15	-0.105
15 μM RA	55.63	-0.065
30 μM RA	54.33	-0.11

Thermal denaturation curves for both WT and the RlinkerA mutant of Ig3-4 are very similar indicating that this mutant does not affect protein stability. T_m is melting temperature and Hill Slope is a measure of cooperativity of unfolding.

Protein Purification



1) Crude lysate
2) Supernatant
3) Flow-through
4) Wash 1
5) Wash 5
6) Elution 1
7) Elution 2
8) TEV digest E1
9) TEV digest E2
10) MW Ladder

Protein purification was assessed at each step using SDS-PAGE gels which separate proteins based on size.

Conclusions

Mutations to the Ig3-4 linker region of palladin lead to significant increases in the beta -sheet structure and decreases in the random coil formation, which undermine the structural integrity of the region, leading to a drastic decline in actin-binding ability.

Acknowledgments

This work is only possible because of the tremendous support from the National Institute of Health (R15GM140422), Beck Lab members, the FYRE in STEM program, and my fantastic lab partner, Nathan.