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What's Linker Got to Do with It? Examining the Structure and Stability of Palladin's Ig3-4 Linker Region

Lauren Hughes, Rachel Sargent, Nathan Ta, Colby Bradford

*Department of Chemistry and Biochemistry, Fairmount College of Liberal Arts and Sciences
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Abstract: Actin is the most abundant protein within all eukaryotic cells and is essential for motility, structure, and cellular division. Actin participates in more protein-protein interactions than any other known protein, and one such relationship involves palladin. Palladin is a lesser-known protein that is typically only expressed during embryonic development. However, recent work has proven that palladin is expressed in metastatic cancer cells. To understand the role palladin plays in cancer metastasis, we must first understand its structure. Palladin is comprised of five immunoglobulin-like domains (Ig), each connected via an unstructured linker region. Our research focuses on one of these linker regions, known as Ig3-4. The Ig3-4 linker consists of forty-one amino acids and is predicted to be intrinsically disordered. Previous research has proven that Ig3 is the minimal actin-binding domain, but binding affinity is significantly increased when the Ig3-4 linker domain is present. However, the Ig3-4 linker has no innate actin-binding ability. Current research seeks to determine the Ig3-4 linker region's effect on palladin and actin's interactions. To determine the structure and function of the Ig3-4 domain, the Beck lab introduced several mutations to the linker. The most prominent mutation is RLinkerA, a conversion of the domain's ten arginines into alanines, which completely disrupted the binding ability of both actin and palladin. All the mutated and wild-type linker regions have undergone circular dichroism spectroscopy to determine structure, as well as chemical and thermal denaturation tests to determine stability. Current analysis of these results indicates no alteration in the structure or stability of either the Ig3 domain or its linker, despite the obstruction of actin-palladin binding.

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