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Introduction

- In North America, cardiac muscle diseases such as heart attacks and myopathies are on the rise.
- Contributing to knowledge in this area, we have focused on a critical muscle protein called titin (connectin) important in force production.
- Changes in the elastic function of cardiac titin regions accompany severe heart failure in humans [1].
- By adjusting the length of titin's extensible region, a muscle can vary its elastic properties [2] and thus passive force capability.
- Previous experiments with muscle fibers showed that titin-based tension is calcium responsive [3].

Purpose

- Identify the potential for passive force regulation of the immunoglobulin (Ig) domain within titin.
- Examine the effect of calcium on the chemical and structural properties of Ig domains.

Experimental Design

- E. Coli used to produce the 27th Ig domain from the I-band region of human cardiac titin. Proteins isolated using his-tagged affinity chromatography.
- Fluorescence spectroscopy was conducted on tryptophan π electrons using a Hitachi F-2000 wavelength scanning spectrofluorimeter at 295nm.

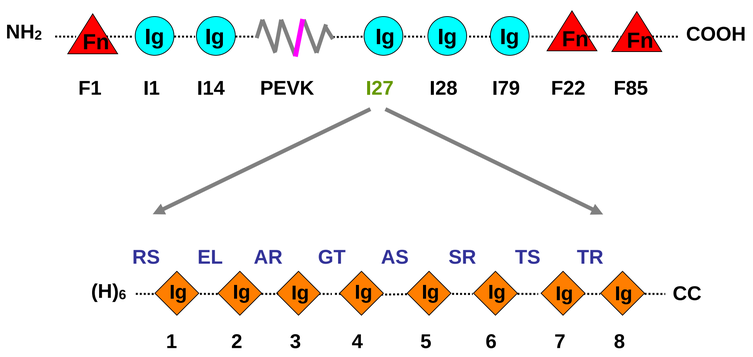

Figure 1: Schematic of titin isoform and arrangement of 8 tandem I27 domains. Ig (I): immunoglobulin; Fn (F): fibronectin; Single letters correspond to their respective amino acid or atomic abbreviations

- Atomic force microscopy (AFM) was performed using a JPK AFM mounted on a Zeiss inverted microscope.

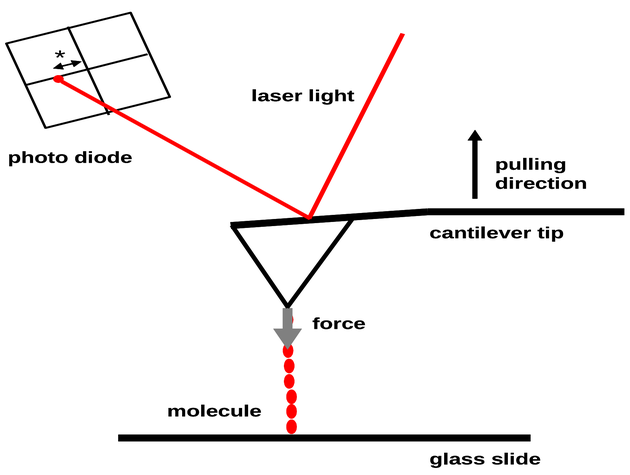

Figure 2: Atomic force microscopy setup. Protein is attached to the substrate via covalent gold-sulfur linkages

Results

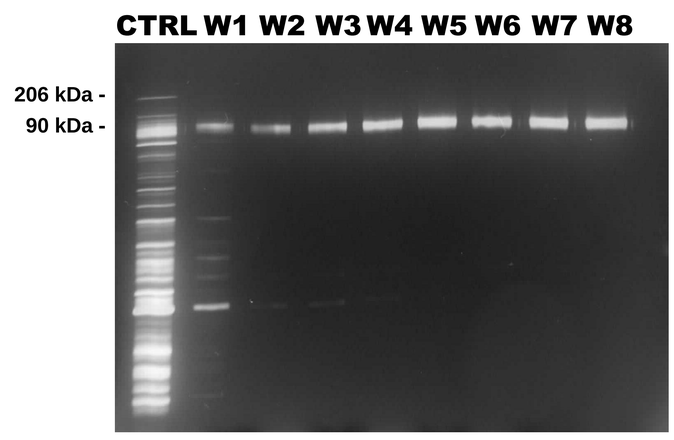

Figure 3: Silver stained SDS-PAGE of I27 domain separation with successively stronger imidazole washes (W)

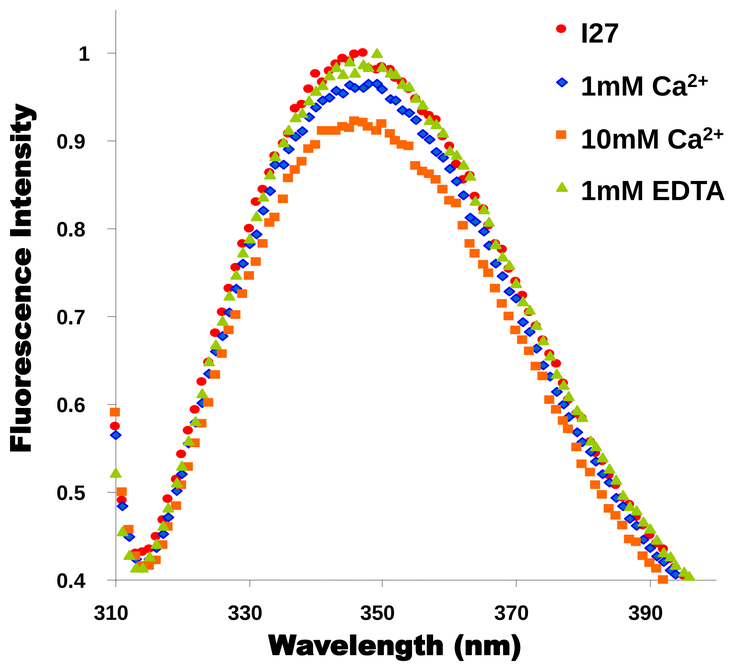

Figure 4: Tryptophan fluorescence emission spectrum for titin I27 excited at 295nm with the addition of calcium (Ca^{2+}) and a calcium chelating agent (EDTA). Note the depression in intensity with increasing [Ca^{2+}] and the partial reversal of this effect with EDTA

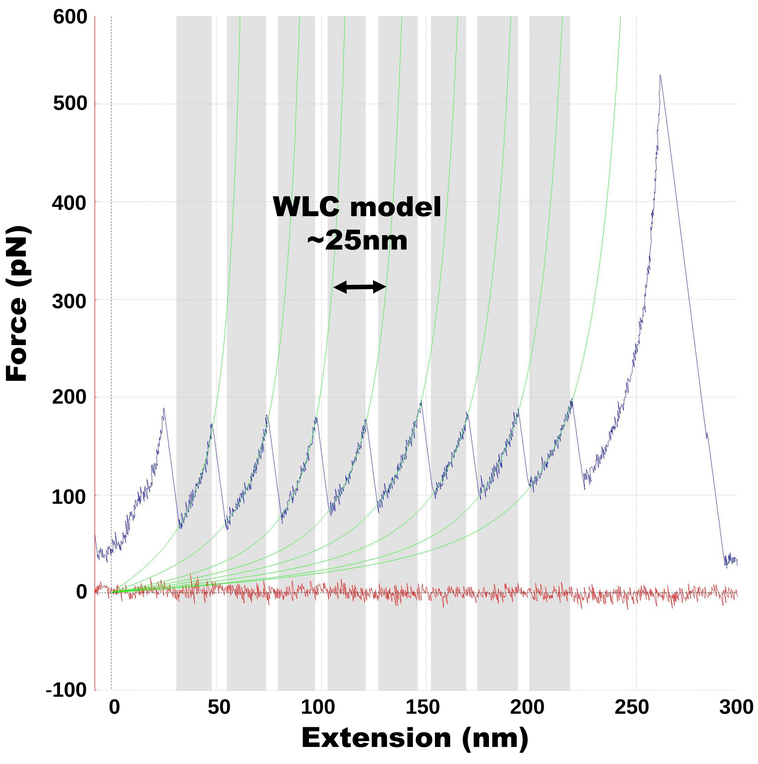

Figure 5: Force curve for I27 in relaxing solution indicating the force attained when the cantilever was extended toward (red) and retracted from (blue) the surface, fit to the Worm-Like Chain Model (green)

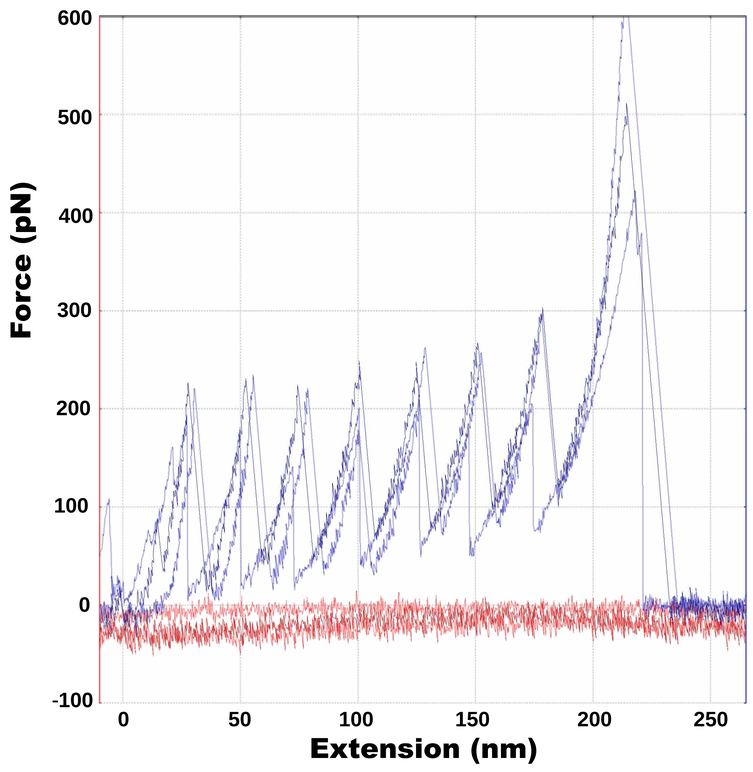

Figure 6: Three overlapping force curves for I27 in the presence of Ca^{2+}. Pulling speed: 1µm/sec, 0.01M [Ca^{2+}]. Note the gradual increase in force with Ca^{2+}

Discussion & Conclusion

- Fluorescence spectroscopy revealed a measurable degree of change in the microenvironment of I27 with the addition of calcium.
- Removing free calcium and repeating the fluorescence experiments lead to the almost complete restoration of fluorescence intensity, indicating reversibility.
- Tryptophan π electron delocalization suggests that calcium interacts and potentially binds to the Ig domain, which may have structural implications for titin's overall stiffness.
- Whether the alteration in the tryptophan environment translates into a change in the mechanical properties of I27, remains to be established.
- Using single molecule AFM, we have the means to evaluate differences in stiffness of Ig domains.
- The application of a mechanical force may trigger the exposure of new binding sites that were buried between domains or in the fold [4].
- Therefore, Ig domain unfolding within titin may modulate its resting length, elasticity and also its ligand binding properties [4].
- This entropic spring can then be used to better understand the changes in passive properties associated with specific cardiac failure.

References

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Acknowledgements

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