Fluorinating the Coiled-Coil Domain of Cartilage Oligomeric Matrix Protein to Study Fiber Design and Assmbly

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Abstract

Rationally designed proteins are gradually becoming more commonplace. Proteins are incredibly varied and diverse in their chemistry, allowing the assembly of rationally designed proteins to be fine-tuned at a molecular level. Over the past decade, considerable efforts have been made to develop protein and peptide based self-assembled systems. The α-helix based coiled-coil proteins systems have been successfully engineered to develop structurally defined fibrils with potential application in nanoelectronics and biomedical field. The α-helical coiled-coil consists of two or more α-helices bound together by non-covalent interaction with a repetitive sequence of hydrophobic and polar residues designated as heptad repeat *abcdefg*. The two rationally designed peptides CC and Q54, derived from Cartilage Oligomeric Matrix Protein, were designed to self-assemble longitudinally based on sticky ends. To improve the thermal and chemical stability of proteins and assembly of fibers we replaced leucine from hydrophobic core with 5,5,5-trifluoro-D-L-leucine (TFL) by residue incorporation. Circular dichroism results indicate that the proteins exhibit a strong α-helical structure in the presence of TFL. Fluorescence microscopy shows the formation of protein fibers in the presence of salt and the small molecule curcumin. The fibers seen in fluorescence microscopy were approximately 600 nm. Transmission electron microscopy (TEM) showed fibers that were 12.6 microns in length and 152.9 nm in width. The incorporation of TFL into α-helical proteins provides a larger insight into the mechanism behind the formation self-assembling nanofibers.

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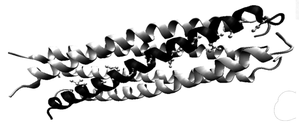
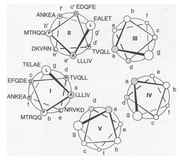
Cartilage Oligomeric Matrix Protein Coiled-Coil (COMPcc)

- N-terminal parallel coiled coil domain
- Characteristic heptad repeat $(a\text{-}g)_n$
- Interactions
  - a,d Hydrophobic
  - g,e Electrostatic
- Homopentamer
  - 7.3 nm x 0.2-0.6 nm hydrophobic pore
  - 1,25-dihydroxyvitamin D_3 (vitamin D), curcumin, all-*trans*-retinol





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Design of Protein Fiber: Q54

Truncated COMPcc (CC)

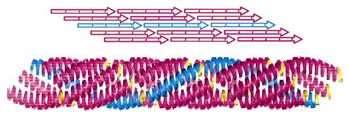
MRGSHHHHHHGSIEGRAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTSKL

Swapped COMPcc about Q54 Position (Q54)

MRGSHHHHHHGSIEGRVKEITFLKNTAPQMLRELQETNAALQDVRELLRQQSKL

APQMLRELQETNAALQDVRELLRQQVKE ITFLKNT APQMLRELQETNAALQDVRELLRQQVKE ITFLKNTSKL

QVKE ITFLKNTAPQMLRELQETNAALQDVRELLRQQSKL APQMLRELQETNAALQDVRELLRQQVKE ITFLKNTSKL



Potekhin et al. *Chemistry & Biology* **2001** 8 1025-1032

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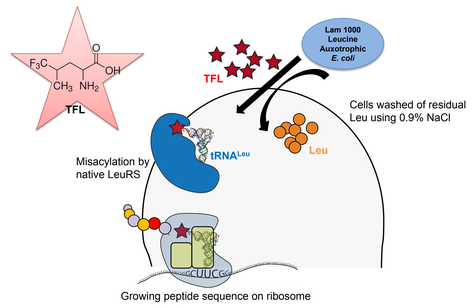
Non-Natural Amino Acid Incorporation



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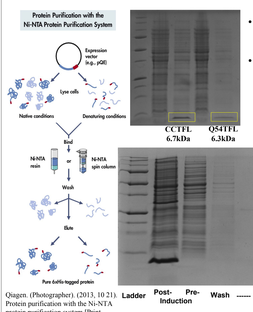
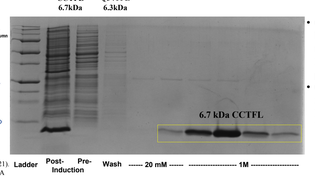
CCTFL Protein Expression and Purification



- Trifluoroleucine incorporation was accomplished after all residual leucine was washed away, and *E. coli cells* were starved of leucine
- IPTG was added to induce expression



- CCTFL containd a hexa-histidine tag, that is used for Ni-NTA resin purification
- CCTFL is eluted in 50 mM Phosphate 1 M Imidazole 6 M Urea pH 8.0 Buffer

Qiagen. (Photographer). (2013, 10 21). Protein purification with the Ni-NTA protein purification system [Print Photo].

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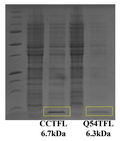
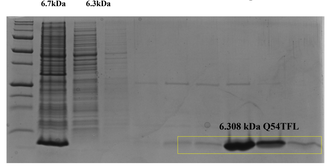
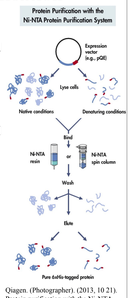
Q54TFL Protein Expression and Purification



- Trifluoroleucine incorporation was accomplished after all residual leucine was washed away, and *E. coli cells* were starved of leucine
- IPTG was added to induce expression
- Q54TFL containd a hexa-histidine tag, that is used for Ni-NTA resin purification
- Q54TFL is eluted in 50 mM Phosphate 1 M Imidazole 6 M Urea pH 8.0 Buffer





Qiagen. (Photographer). (2013, 10 21). Protein purification with the Ni-NTA protein purification system [Print Photo].

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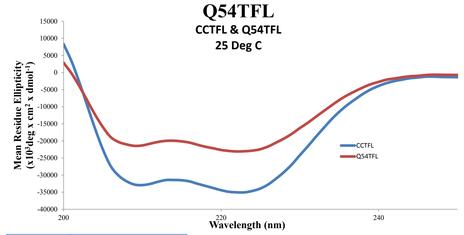
Circular Dichroism Comparasion of CCTFL & Q54TFL



$\theta_{222}/\theta_{208}$	50 mM Phosphate Buffer 100 mM NaCl pH 8.0
CCTFL	1.10169
Q54TFL	1.09805

Circular dichroism is used to confirm the secondary structure of the proteins. The minima at $\Theta_{222}/\Theta_{208}$ is characteristic of α-helicity. The closer the value is to 1, the more α-helical the protein

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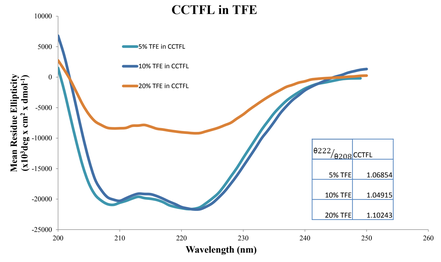
CD of CCTFL in TFE



$\theta_{222}/\theta_{208}$	CCTFL
5% TFE	1.06854
10% TFE	1.04915
20% TFE	1.10243

The addition of varying amounts of TFE (Trifluoroethanol) to the proteins assists in the formation of protein fibers. The CD spectra measured was in 50 mM Phosphate 100 mM NaCl pH 8.0 Buffer.

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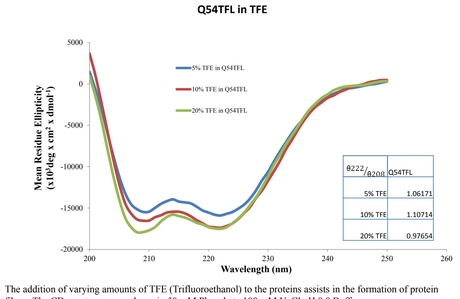
CD of Q54TFL in TFE



$\theta_{222}/\theta_{208}$	Q54TFL
5% TFE	1.06171
10% TFE	1.10714
20% TFE	0.97654

The addition of varying amounts of TFE (Trifluoroethanol) to the proteins assists in the formation of protein fibers. The CD spectra measured was in 50 mM Phosphate 100 mM NaCl pH 8.0 Buffer.

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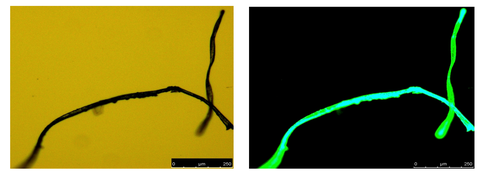
Fluorescence Microscopy



After the structure of the proteins was confirmed using circular dichroism. 50 µL of 20 µM CCTFL was mixed with 50 µL of 100 µM circumin: an effective concentration of 10 µM CCTFL 50 µM curcumin. After a brief incubation period fluorescence microscopy was utilized to assess fiber formation
Image on the left shows the mixture described above at 20x-phase contrast
Image on the right shows the mixture described above at 20x-fluorescence

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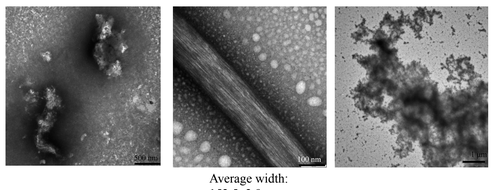
Transmission Electron Microscopy



Average width:
152.5±2.9

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Conclusion and Future Work

- Fluorinated CC & Q54 provide a greater insight into the folding mechanisms of protein
- Preliminary fluorescence microscopy shows the addition of circumin also affects fiber formation
- Preliminary TEM shows the addition of TFE also has an affect on fiber formation
- Future work includes the testing of different salts to determine their affects on fiber formation

Montclare Lab.

Acknowledgements:

This work is supported by the Army Research Office W911NF-11-1-0449 and in part by the MRSEC Program of the National Science Foundation under Award Number DMR-0820341.

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