Impact of Fluorinated Amino Acids on Artificial Protein Block Copolymers of Two Self-assembling Domains

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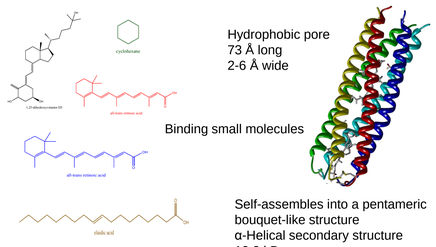
1 Abstract

The requirement for smart protein-derived biomaterials to change in macromolecular structure in response to external stimuli necessitates the design of controllable modes of self-assembly. The recent advances in unnatural amino acid incorporation enables the integration of chemical diversity into such proteins, further expanding the level of control and materials properties. In particular, fluorinated amino acids have been employed in protein design to improve stability against heat and chemical denaturants. Here, we describe our progress towards fabricating fluorinated protein block polymers and examining the impact of fluorination on the physico-chemical properties of the biomaterials. Specifically, we have incorporated *para*-fluorophenylalanine and trifluoroleucine into three block polymers that consist of a β-spiral elatin-mimetic protein (E) and an α-helical coiled-coil region of cartilage-oligomeric matrix protein (C). These proteins, synthesized as the block sequences – EC, CE, and ECE – are chosen for their distinct structures, functions, and modes of self-assembly. We demonstrate successful incorporation of the non-natural amino acids as well as characterization emphasizing their structural and functional distinction relative to the non-fluorinated constructs.

2 Cartilage Oligomeric Matrix Protein Coiled-Coil (C)



Hydrophobic pore
73 Å long
2-6 Å wide

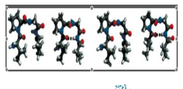
Binding small molecules

Self-assembles into a pentameric bouquet-like structure
α-Helical secondary structure
10.9 kDa

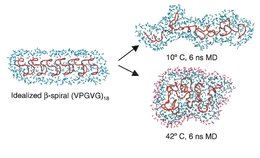
V. N. Malashkevich, R. A. Kammerer, V. P. Efimov, T. Schulthess, and J. Engel, *Science*, (**1996**) *274*, 761-765.
Suat Ozbek, Jürgen Engel, Jörg Stetefeld, *EMBO J* **2002**, *21*, 5960-8.

3 Elastin (E)

(Val/Ile)-Pro-Xaa-Yaa-Gly



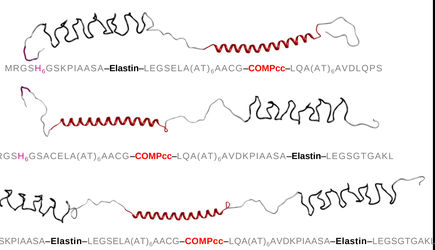
Lower criticial solution temperature (LCST) – from liquid to solid by adding heat



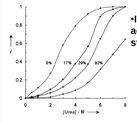
- **Temperature-sensitive aggregation and self-assembly**
- **Coupling to other functional domains has been shown to affect both aggregation and temperature-sensitivity**
- **Focus of potential use lies in drug (small molecule) delivery and release**

B. Li, V. Daggett, *J Muscle Res Cell Motil* **2002**, *23*, 561-73.
Bumjoon Kim, Ashutosh Chilkoti, *J Am Chem Soc* **2008**, *130*, 17867-73.

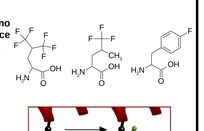
4 Protein Block Polymers



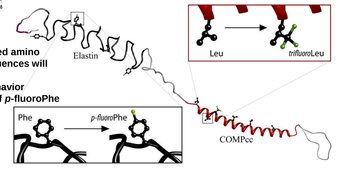
5 Non-canonical Amino Acids



- **Incorporated non-canonical amino acids have been shown to enhance structural stability some proteins**
  - **Hexafluoroleucine**
  - **Trifluoroleucine**
  - ***p*-fluorophenylalanine**

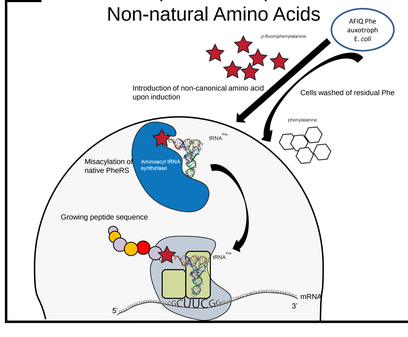


- **We hypothesize that the incorporation of fluorinated amino acids into our fusion sequences will result in an alteration of temperature-sensitive behavior**
- **Separate incorporation of *p*-fluoroPhe and trifluoroLeu**



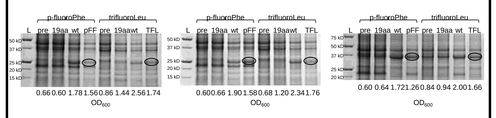
Tang, Y. et al. Fluorinated Coiled-Coil Proteins Prepared In Vivo Display Enhanced Thermal and Chemical Stability, *Angew. Chem. Int. Engl* **40**, 1494-1496(2001).
(1)A James Link, David A Tirrell, *Methods* **2005**, *36*, 291-298.

6 Residue-specific Incorporation of Non-natural Amino Acids

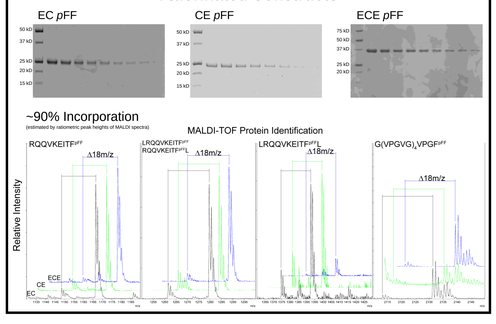


7 Expression

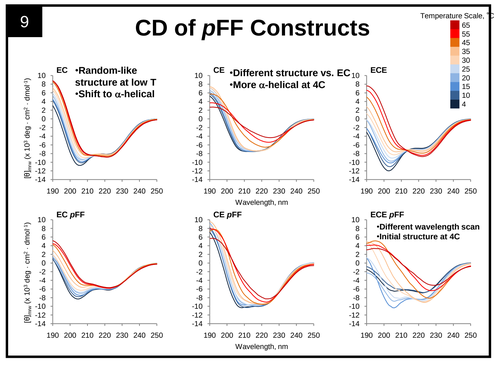
- **Small-scale(5 mL) and large-scale (100 mL) expressions of**
  - **Phe auxotrophic AFIQ cells**
  - **Leu auxotrophic LAM1000 cells**
- **Residual natural amino acids removed prior to induction with IPTG by washing with 2 cycles of washing with 0.9% NaCl**
- **All lanes in gels below normalize to OD_{600}=1.00**



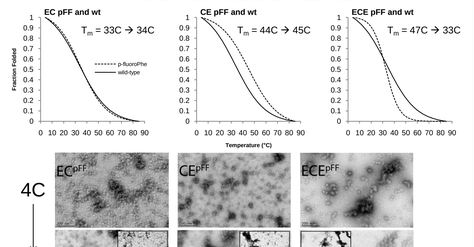
8 Purification and Identification of Fluorinated Constructs



9 CD of *p*FF Constructs

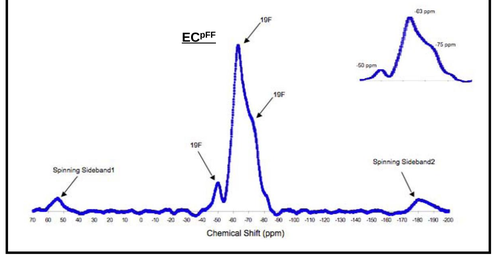


10 Comparison of Melting Curves of Wild-type and Fluorinated Constructs



11 ^{19}F NMR of Fluorinated Sample

- **Precursor to potential MRI imaging studies**
- **Performed on EC^{pFF} sample~ 6mg**



12 Conclusions & Future Work

- Successful incorporation of *p*FF into elastin/COMPcc fusions
- Difference in melting curves apparent in fluorinated constructs
  - Increase in CE cooperativity, demonstrating a dependence on block orientation when comparing fluorinated proteins
  - Increase in ECE cooperativity but decrease in T_m, suggesting an accelerated hydrophobic effect
- Microscopy studies of protein solutions to investigate supramolecular assembly structures

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