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

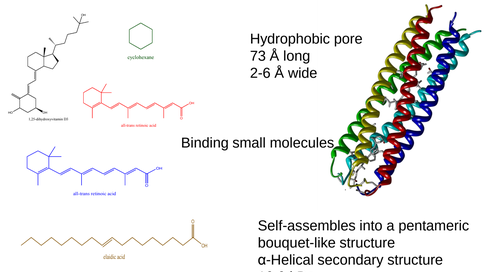
Carlo Yuvienco[1], Jennifer S. Haghpanah[1], Richard Hwang and Jin Kim Montclare[1,2]

Chemical & Biological Sciences, Polytechnic Institute of NYU, Brooklyn, NY, 11201[1]
Department of Biochemistry, SUNY Downstate Medical Center, Brooklyn, NY, 11203 [2]

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 `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

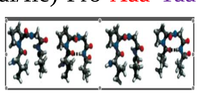
Binding small molecules

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

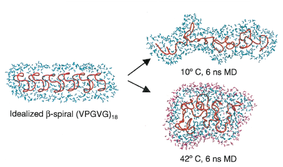
V. N. Malashkevich, R. A. Kammerer, V. P. Efimov, T. Schulthess, and J. Engel, *Science*, (**1996**) *274*, 761-765.
Suat Ozbek, Jürgen Engel, Jörg Stetefeld, *EMBO J* **2002**, *21*, 5960-8.

3 Elastin (E)

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



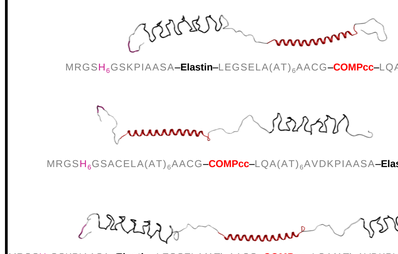
Lower criticial solution temperature (LCST) – from liquid to solid by adding heat



- **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**

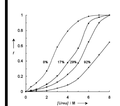
B. Li, V. Daggett, *J Muscle Res Cell Motil* **2002**, *23*, 561-73.
Bumjoon Kim, Ashutosh Chilkoti, *J Am Chem Soc* **2008**, *130*, 17867-73.

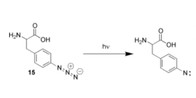
4 Protein Block Polymers



Elastin = [(VPGVG)$_2$VPGFG(VPGVG)$_2$]$_{16}$VP
COMPcc = DLAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDASG

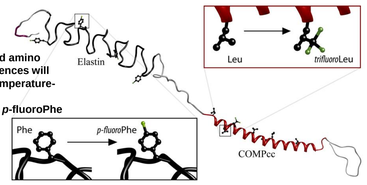
5 Non-canonical Amino Acids





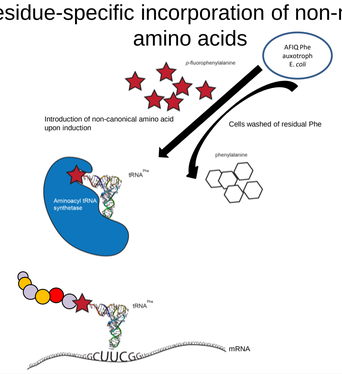
- **Incorporated non-canonical amino acids have been shown to enhance structural stability some proteins**
- **Photo-crosslinking possible with the incorporation of *p*-azidoPhe**

- **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**



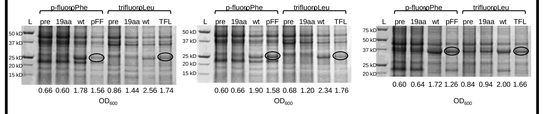
Tang, Y. et al. Fluorinated Coiled-Coil Proteins Prepared In Vivo Display Enhanced Thermal and Chemical Stability. *Angew. Chem. Int. Ed. Engl* **40**, 1494-1496(2001).
[1]A James Link, David A Tirrell, *Methods* **2005**, *36*, 291-298.

6 Residue-specific incorporation of non-natural amino acids



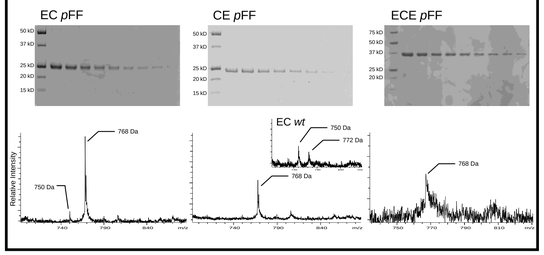
7 Expression

- **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**

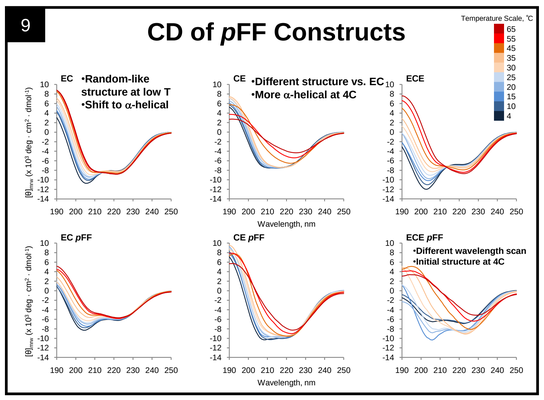


8 Purification of *p*FF Constructs

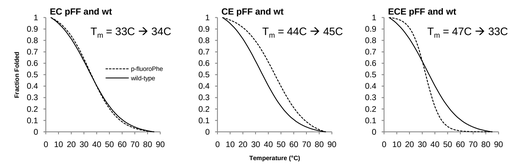
- **Purification of whole cell lysate under denatured conditions (6M urea)**
- **Co^{2+} affinity chromatography using a 5mL HiTrap IMAC FF column (GE)**
- **Gels showing elution fractions from purification**
- **MALDI-TOF analysis confirming non-canonical amino acid incorporation**



9 CD of *p*FF Constructs



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



- **Negligible effect on melting temperature for EC and CE diblocks**
- **Notable shift in T_m for ECE triblock**
- **Enhanced cooperativity of ECE with incorporated fluorinated Phe**
- **Though melt temperatures are the same as wild-type, the CE shows an increase in transition cooperativity versus the EC diblock upon incorporation**

11 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
- We will continue to incorporate other non-canonical aa into the fusions, including trifluoroLeu, photoLeu and *p*-azidoPhe
- Microscopy studies of protein solutions to investigate supramolecular assembly structures

ACKNOWLEDGEMENTS

WE WOULD LIKE TO THANK POLYTECHNIC UNIVERSITY START-UP FUNDS, THE OTHMER INTITUTE, THE WECHLER AWARD, AIR FORCE OFFICE OF SCIENTIFIC RESEARCH, SOCIETY OF PLASTIC ENGINEERS, ACS CHEMISTRY INSITUTE, ACS ENVIORNMENTAL CHEMISTRY DIVISION, UNILEVER, THE NATIONAL SCIENCE FOUNDATION GK-12 FELLOWS GRANT DGE-0741714