Orientation and number of blocks influence structure and self-assembly of protein copolymers

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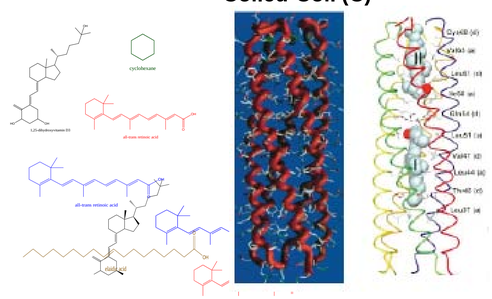
1 Abstract

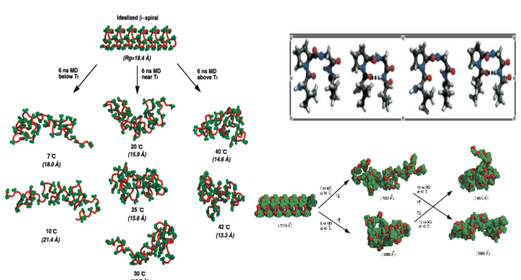
The requirement for smart biomaterials to change in macromolecular structure in response to external stimuli necessitates the design of controllable modes of self-assembly. Driven by this need and inspired by the natural self-assembly of proteins, we describe the biosynthesis and characterization of three block polymers that consist of a β-spiral elastin-mimetic polypeptide (E) and the α-helical coiled-coil region of cartilage-oligomeric matrix protein (C). These proteins, synthesized as the block sequences – EC, CE, and ECE – were chosen for their distinct structures, functions, and modes of self-assembly. For these fusion constructs we demonstrate that the block orientation and the number of repeated blocks of the two protein motifs play a significant role in their self-assembly on the micro- and macroscale. Our results provide insight into the future development of smart biomaterials with emergent properties.

2 Cartilage Oliogmeric Matrix Protein Coiled-Coil (C)



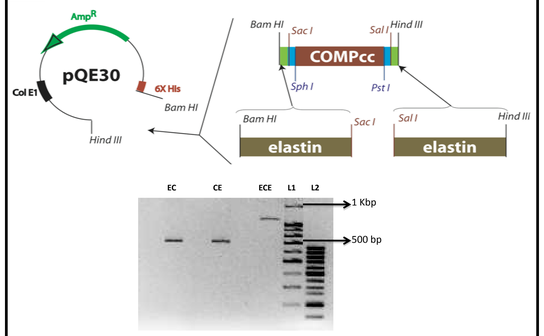
V. N. Malashkevich, R. A. Kammerer, V. P. Efimov, T. Schulthess, and J. Engel, *Science*, (**1996**) *274*, 761-765.

3 Elastin (E)

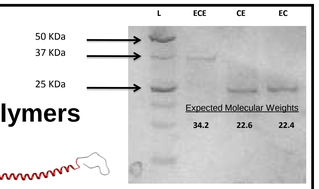


Li, B.; Alonso, D. O.; Daggett, V., The molecular basis for the inverse temperature transition of elastin. *J Mol Biol* **2001,** 305, (3), 581-92.

4 Cloning of Block Co-Polymers



5 Protein Block Polymers



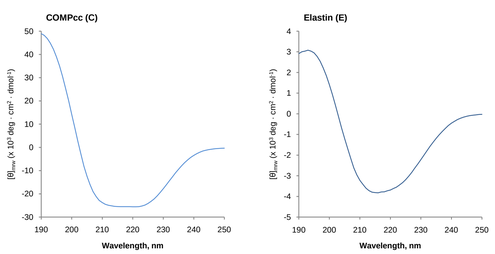
MRGSH$_6$GSKPIAASA–**Elastin**–LEGSELA(AT)$_6$AACG–**COMPcc**–LQA(AT)$_6$AVDLQPS

MRGSH$_6$GSACELA(AT)$_6$AACG–**COMPcc**–LQA(AT)$_6$AVDKPIAASA–**Elastin**–LEGSGTGAKL

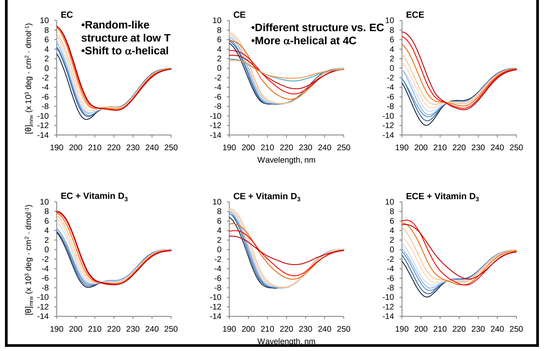
MRGSH$_6$GSKPIAASA–**Elastin**–LEGSELA(AT)$_6$AACG–**COMPcc**–LQA(AT)$_6$AVDKPIAASA–**Elastin**–LEGSGTGAKL

Elastin = [(VPGVG)$_2$VPGFG(VPGVG)$_2$]$_5$VP
COMPcc = DLAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDASG

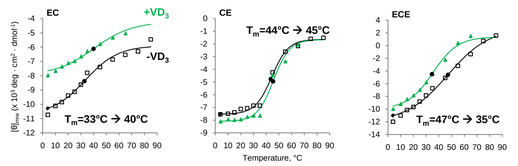
6 Circular Dichroism (CD) of Homopolymers



7 CD of Block Polymers

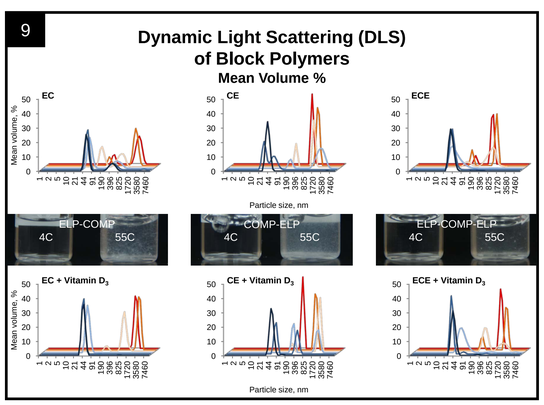


8 Comparison of Melting Curves in the Presence and Absence of Vitamin D_3

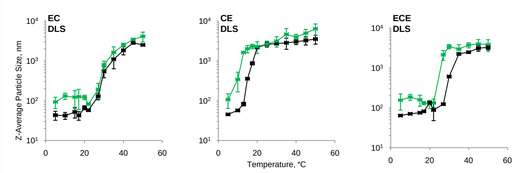


- **Added thermal stability – less random conformation – in the presence of vitamin D_3 for EC**
- **Presence of additional E domain increases T_m – diblocks vs. triblock**
- **Enhanced cooperativity in the presence of vitamin D_3 for ECE**
- **The influence of the small molecule on the polymer structure and assembly is dependent on the block orientation and composition**

9 Dynamic Light Scattering (DLS) of Block Polymers

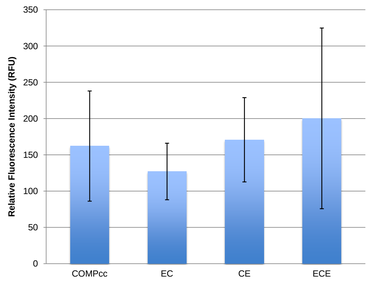


10 Comparison of LCST in the Presence and Absence of Vitamin D_3



- **Low temperature values consistent with overall lengths and molecular weights**
- **Identical T_t for EC and ECE suggests that aggregation behavior is dependent on the N-terminal block**
- **C-terminal elastin (CE) promotes enhanced aggregation when incubated with vitamin D_3**

11 Binding of Polymers to All-Trans Retinol



12 Conclusions & Future Work

- Our studies with CD and DLS suggest that the physicochemical behaviors of EC and CE constructs, compositionally similar macromolecules, are different.
- Further comparison with ECE shows that the number of blocks contributes to modes of self-assembly taking place.
- We will continue to study rheological properties of the constructs.
- We hope these block co-polymers will provide protein engineers with an extra level of control for drug delivery. These will also serve as novel scaffolds for tissue engineers.

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