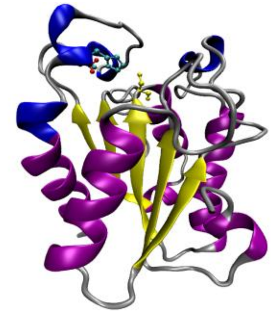
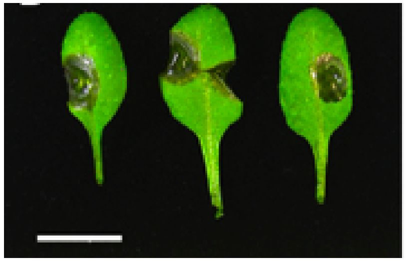
Nanoscaled Biocatalysts and Self-Assembling Protein Polymers

Jin Kim Montclare
Polytechnic Institute of NYU
Department of Chemical and Biological Sciences
and SUNY Downstate Medical Center
Department of Biochemistry
Biomedical Engineering

IGERT MassNanoTech UMASS Amherst March 4, 2010

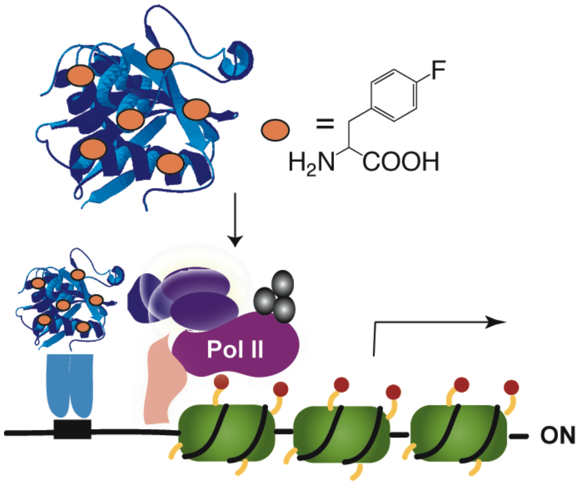
Protein Engineering and Molecular Design





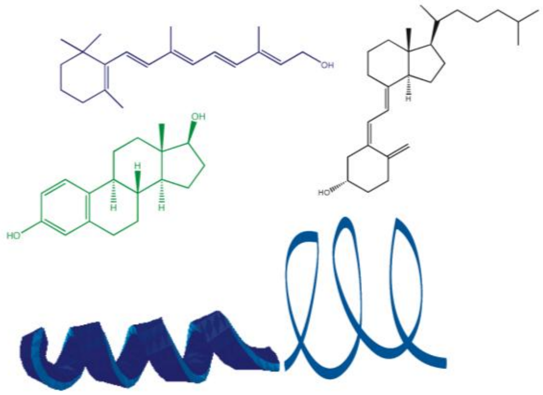
Enzymes for Polymer Modification

Montclare *et. al. JACS* **2009** *131, 15711,* Baker & Montclare *Polymer Biocatalysts and Biomaterials,* **2010**.



Control reactivity for target substrate

Montclare *et. al. Biotech Bioeng* **2006** *94,* 921; Montclare *et. al. BMCL* **2007** 17, 5907; Montclare *et. al. BMCL* **2009** 19, 5449; Voloshchuk & Montclare *MolBioSys* **2010** 6, 65.



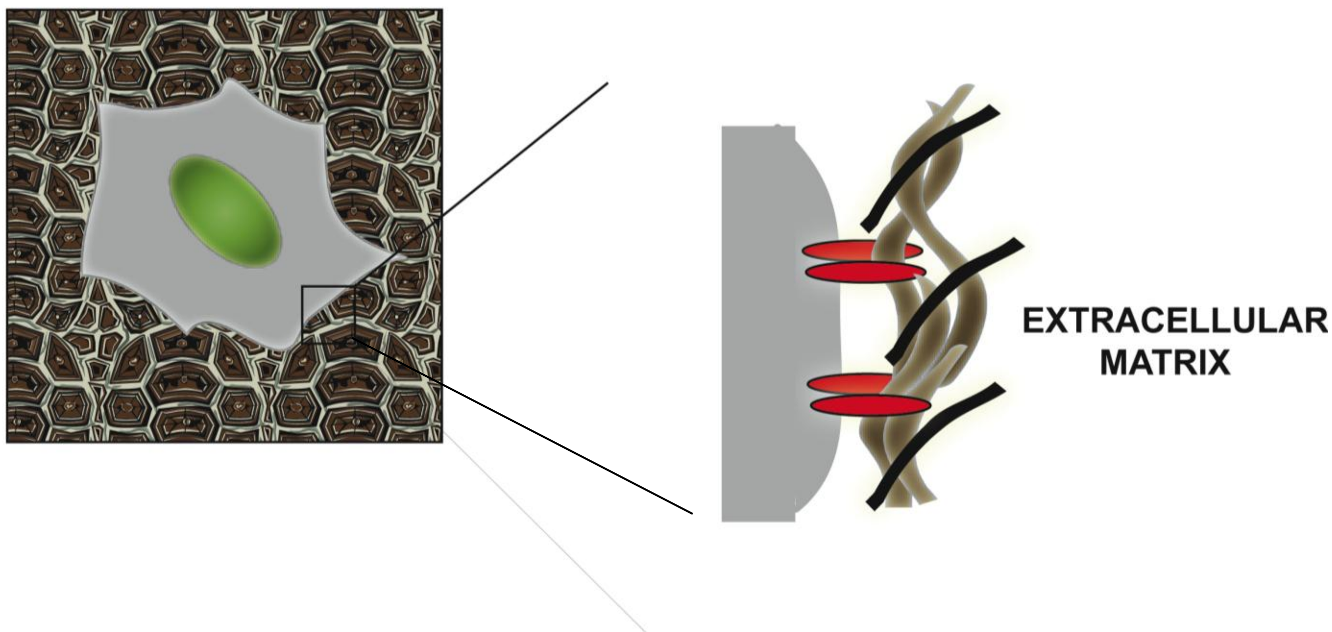
self-assembly

Biosynthesis of protein materials

Montclare *et. al. Polymer Biocatalysts and Biomaterials,* **2008***;* Montclare *et. al. Polymers Adv. Tech.* **2008** 19, 454; Montclare *et. al. ChemBioChem* **2009** 10, 2733; Montclare *et al. Biochemistry* **2009** 48, 8559; Montclare *et. al*. **2010** *MolBioSys under revision.*

Protein Interactions: Extracellular Matrix

Structural role



Question: Inspired by extracellular matrix proteins, can we engineer novel biomaterials with defined structural properties?

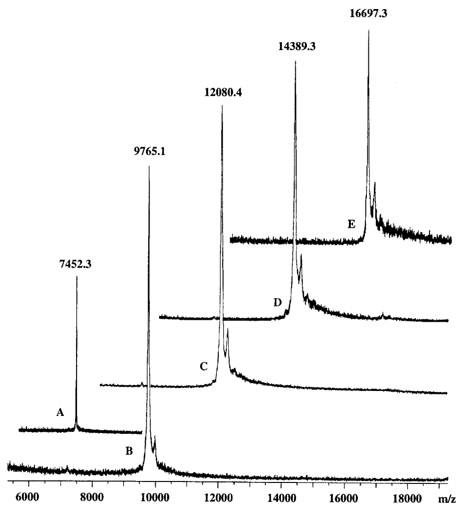
Biopolymer Synthesis

Chain length, sequence and stereochemistry

- **Mono-dispersity**
- **Well defined secondary structures**

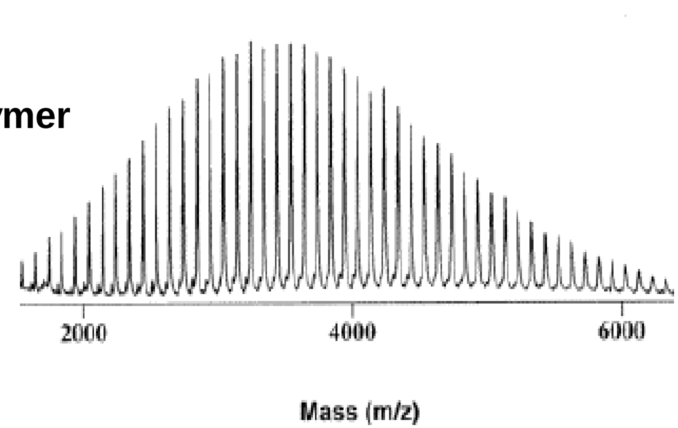
Proteins

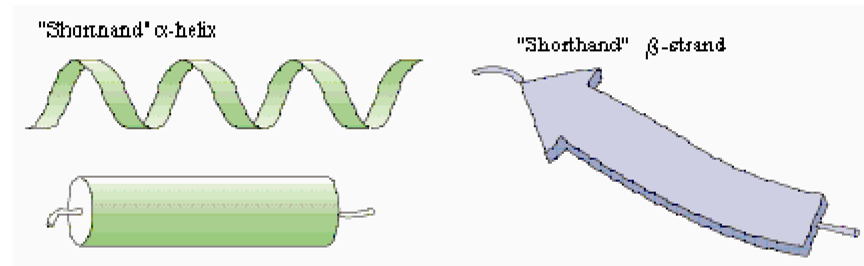
(PLGAs)



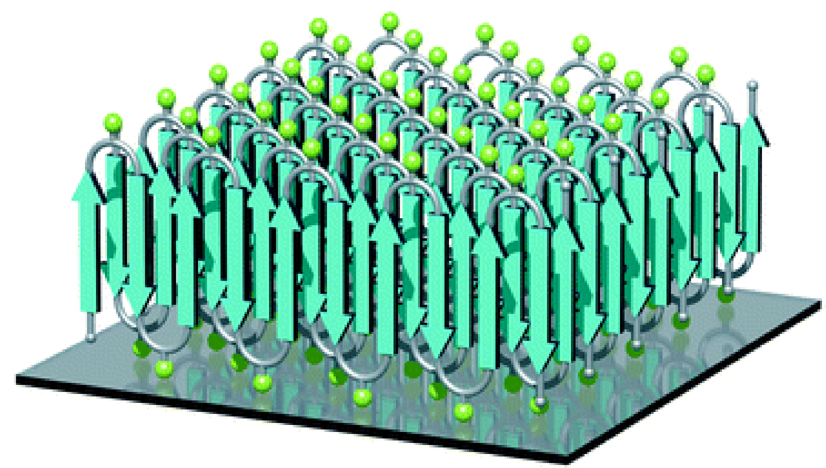
Synthetic Polymer

(PMMA)





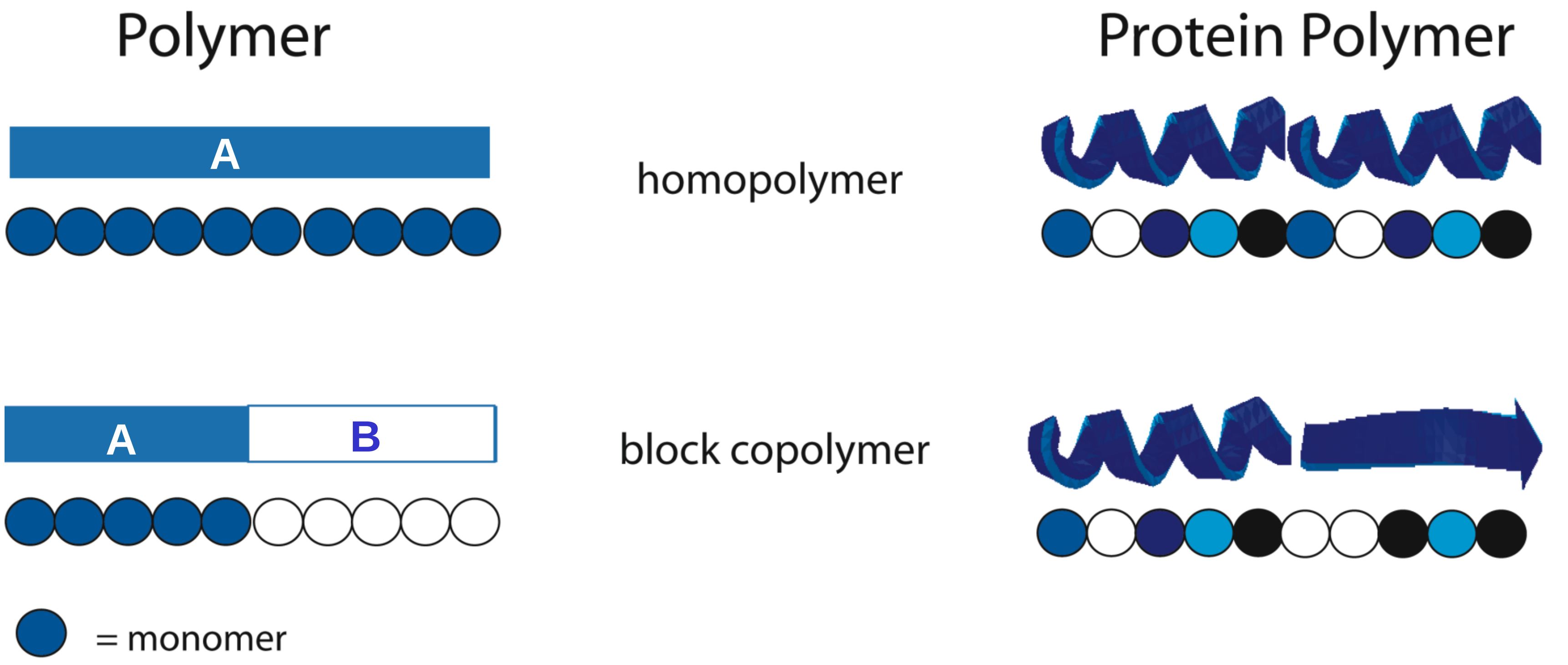
$[(AG)_xEG]_n$



I. Chen and B. Gao *Anal. Chem*., 1997, **69**, 4399

Krejchi et.al. *Science*, 1994, **265**, 1427

Conventional Polymer vs. Protein Polymer

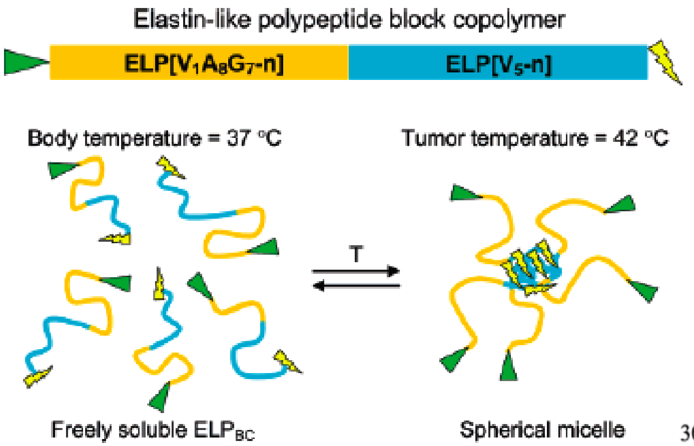


Polymers: comprised of repeated monomers

Protein Polymer: comprised of motifs with a particular monomer sequence

Examples of Protein Polymers: Elastin and Helix

Elastin-elastin

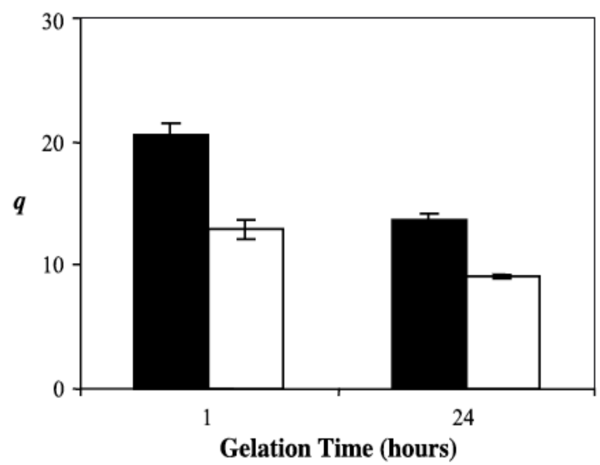


Chilkoti et.al. *JACS*, 2008, **130**, 687

Most block polymers bear similar secondary structure or one structured and one unstructured domain.

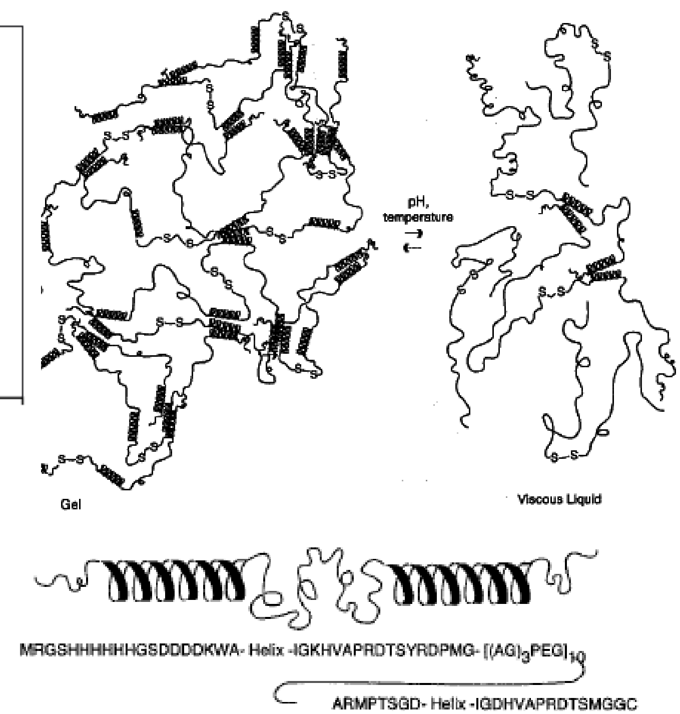
Silk-elastin

SELP hybrid



Ghandehari et.al. *Biomac*, 2003, **3**, 602

Helix-random coil



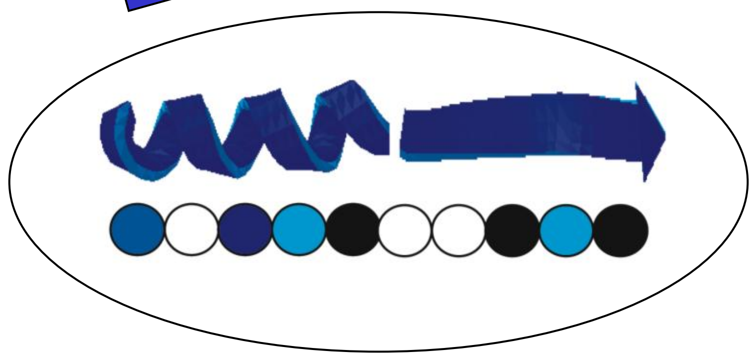
Tirrell et.al. *Science*, 1998, **281**, 389

Block polymers of 2 different self-assembling domains (SADs)

Actuation

Structured templates

Delivery/ sequestration dynamic scaffold



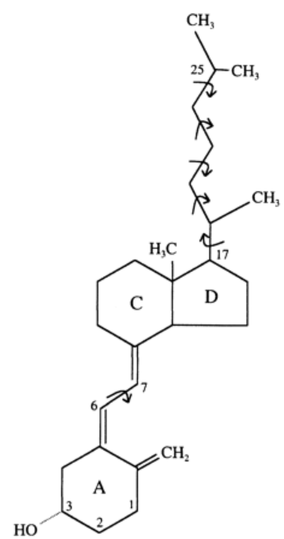
Simple models for protein folding

tunable smart-materials

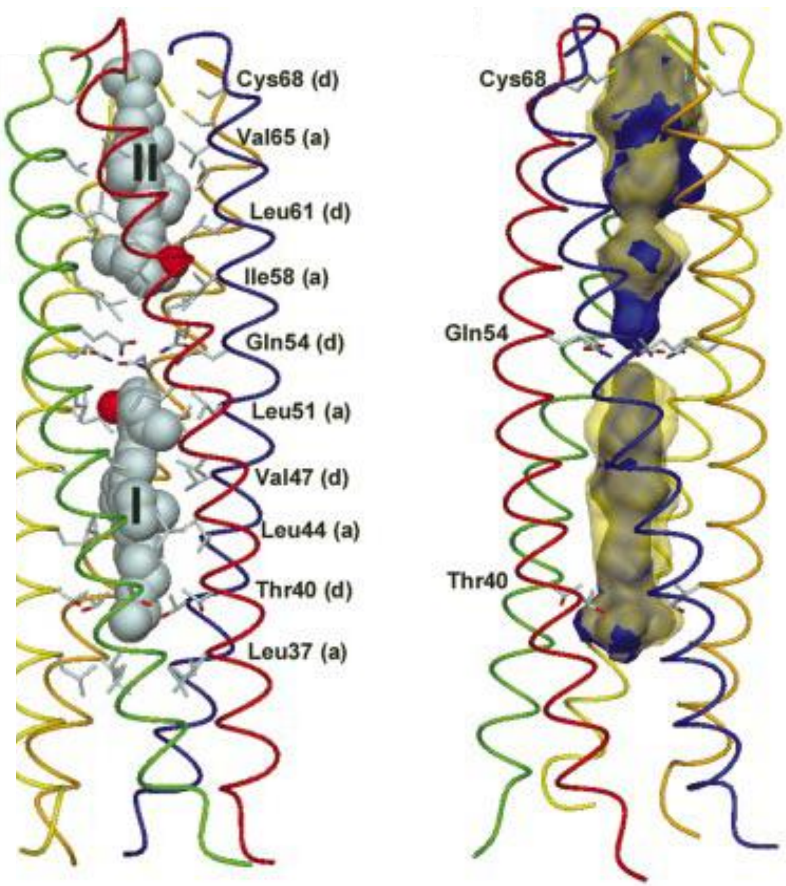
Understand self-assembly, control hierarchical organization

Cartilage oligomeric matrix protein coiled coil (COMPcc)

- Comprised of homopentamer of coiled coils
- Hydrophobic pore 7.3 nm long and 0.2-0.6 nm diameter
- Binds the hormone 1,25-dihydroxy (vitamin D3)

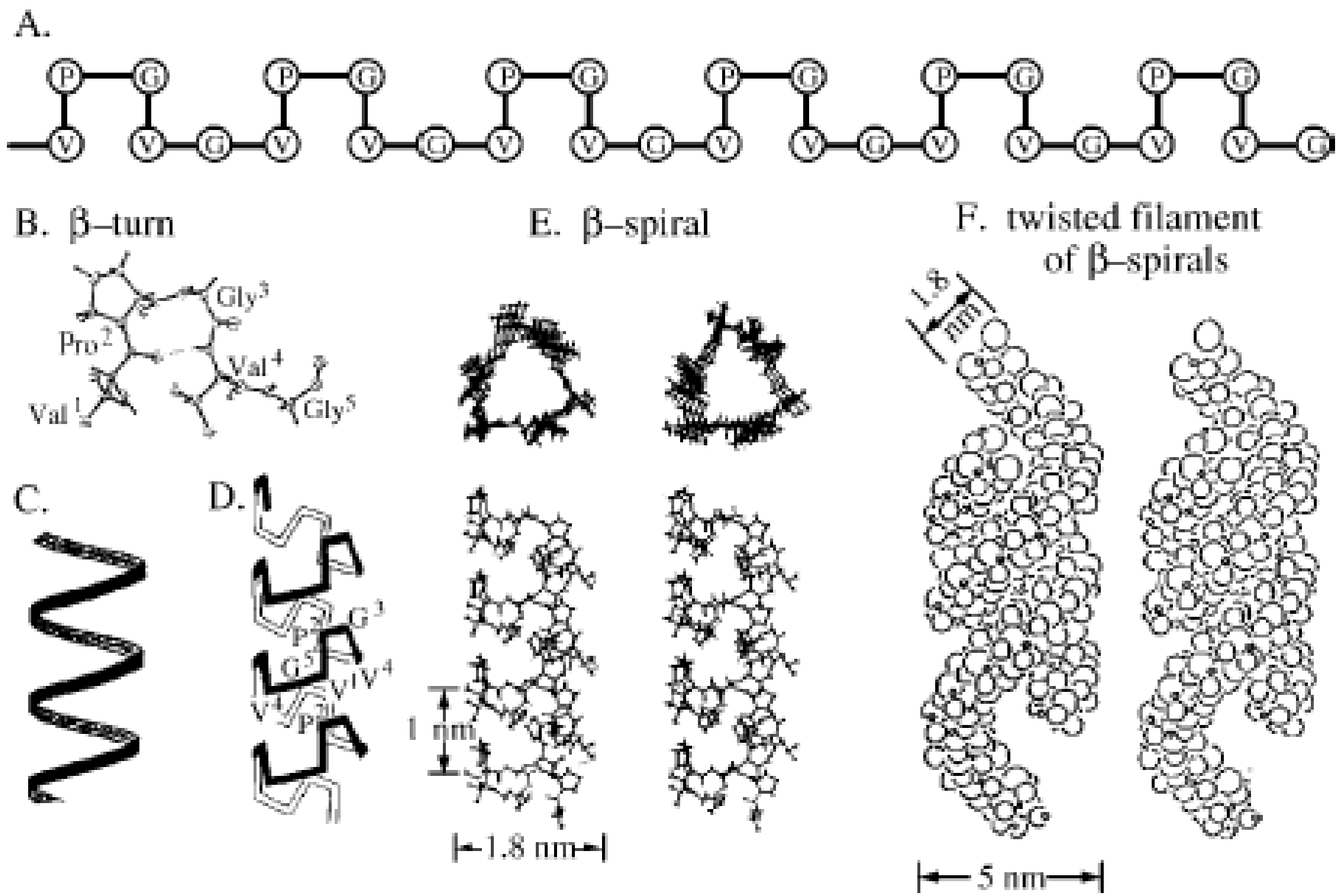


Vitamin D3



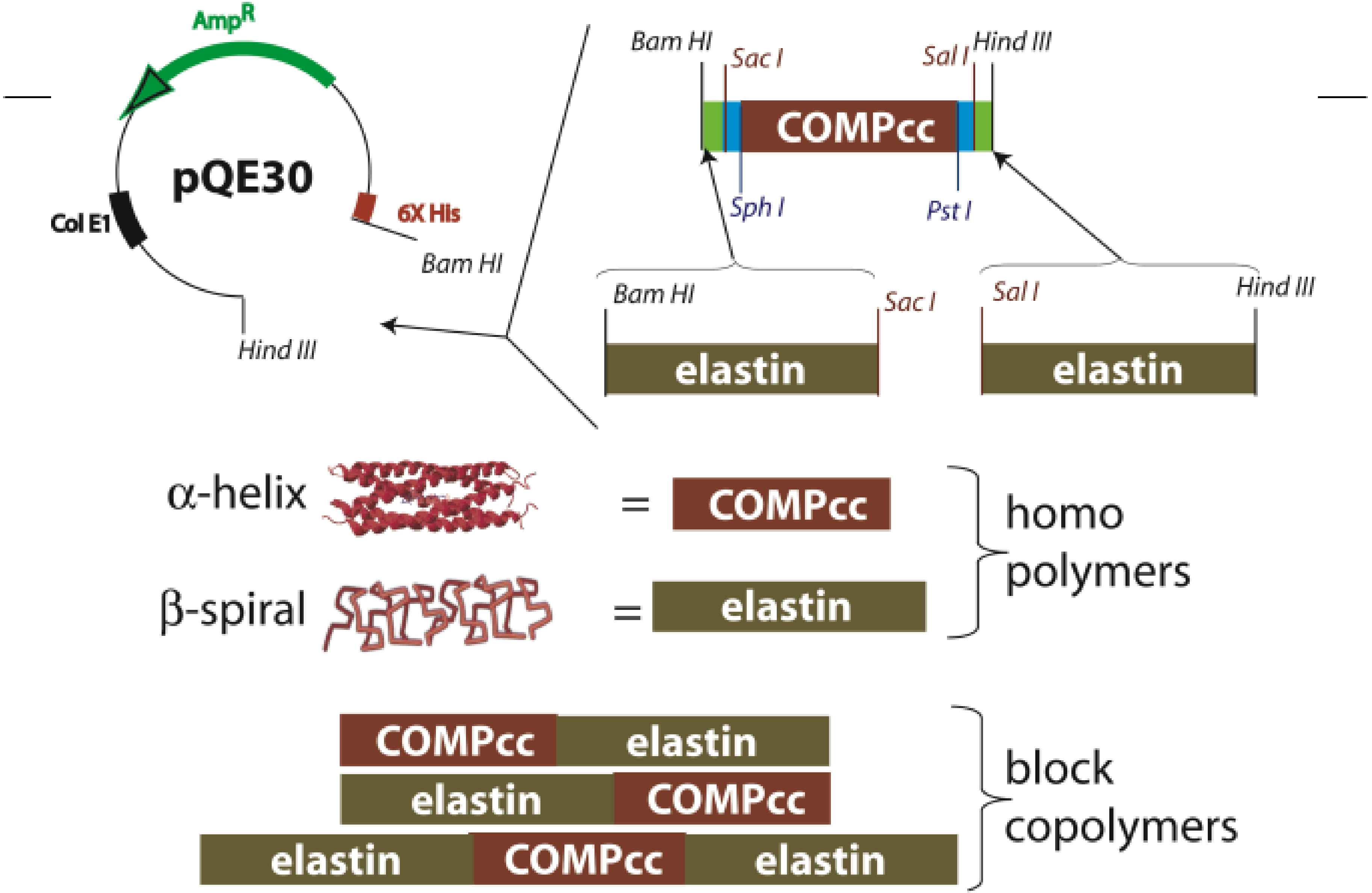
Ozbeck, S., Engel, J., Stetefeld, J. *EMBO J.* **2002, *21*,** 5960.

Elastin polypeptide



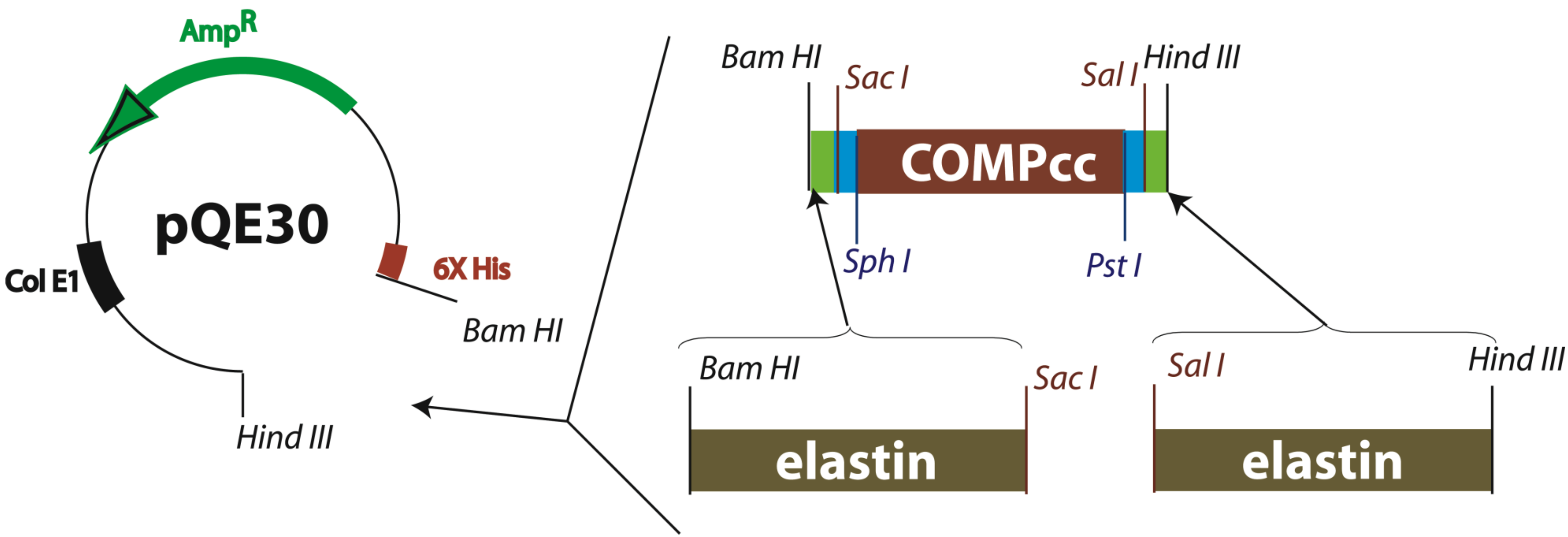
- Comprised of pentapeptide repeat (GVP**X**P)$_n$
- Exhibits lower critical solution temperature (LCST) depends on identity of **X** and number of repeats

Urry, D.W. and Parker, T.M. *J. Muscle Res Cell Mot.* **2002,** *23,* 543.



Question: Does orientation of blocks and number of blocks influence assembly and structure?

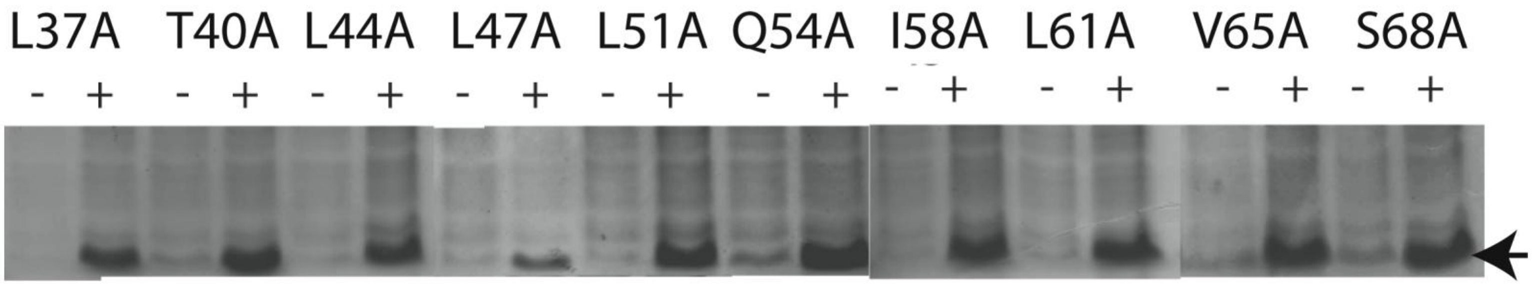
A Modular Approach

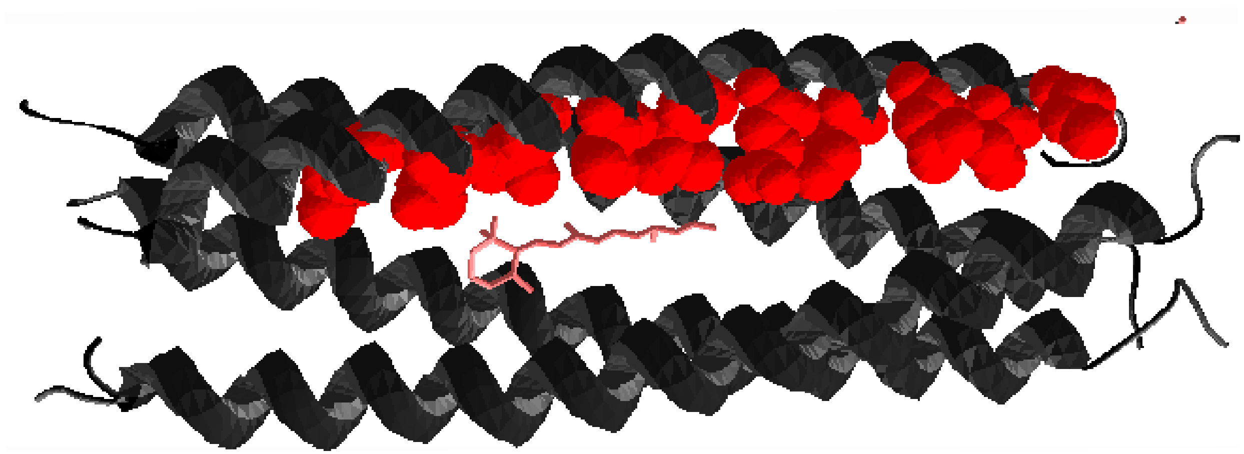


added a linker as distance to prevent interference on COMPcc stability from direct fusion

Single-Alanine Mutants of COMPcc

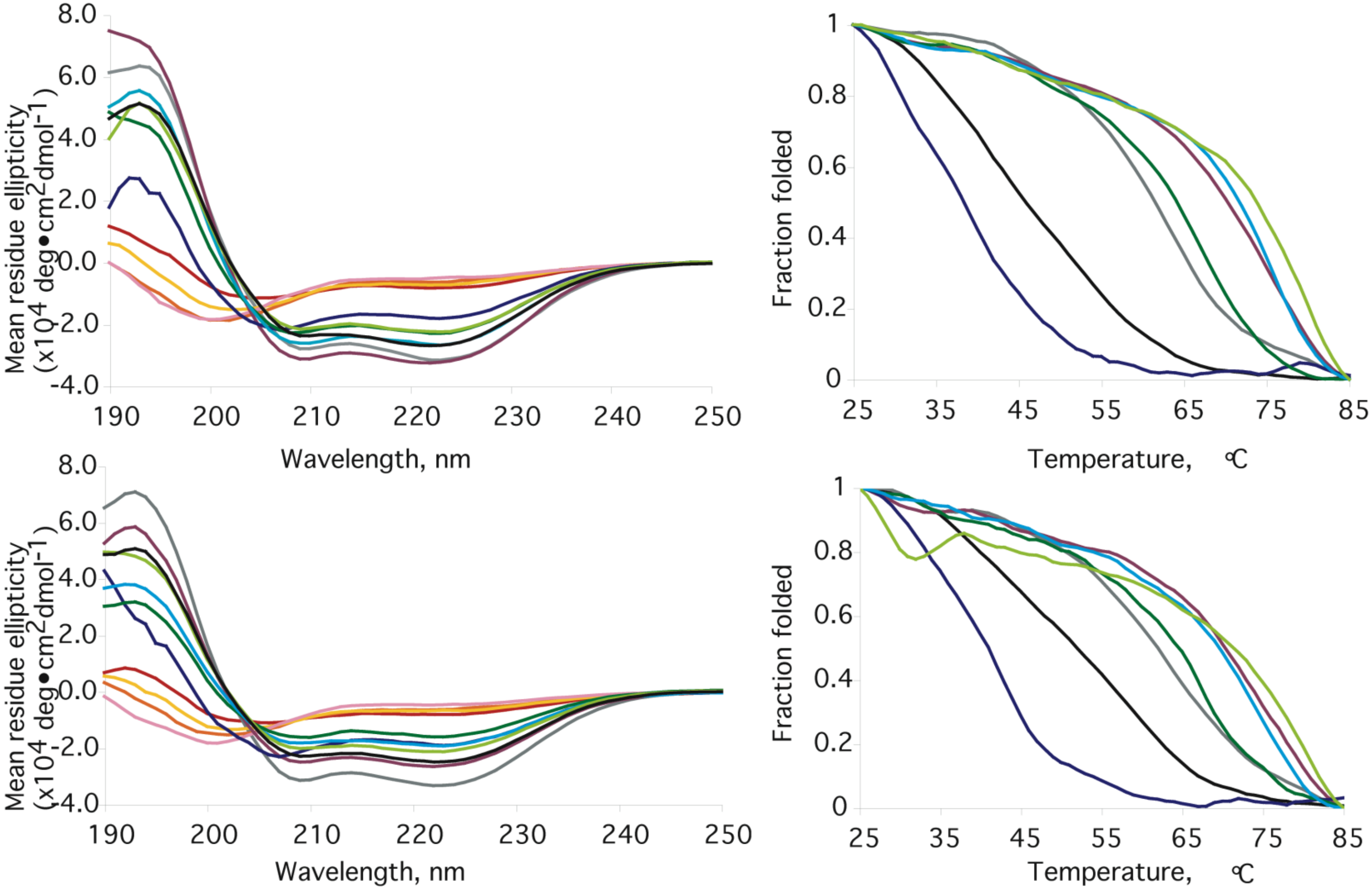
DLAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDASG





S. Gunasekar,M. Asnani, C. Limbad, W. Hom

Characterization via CD



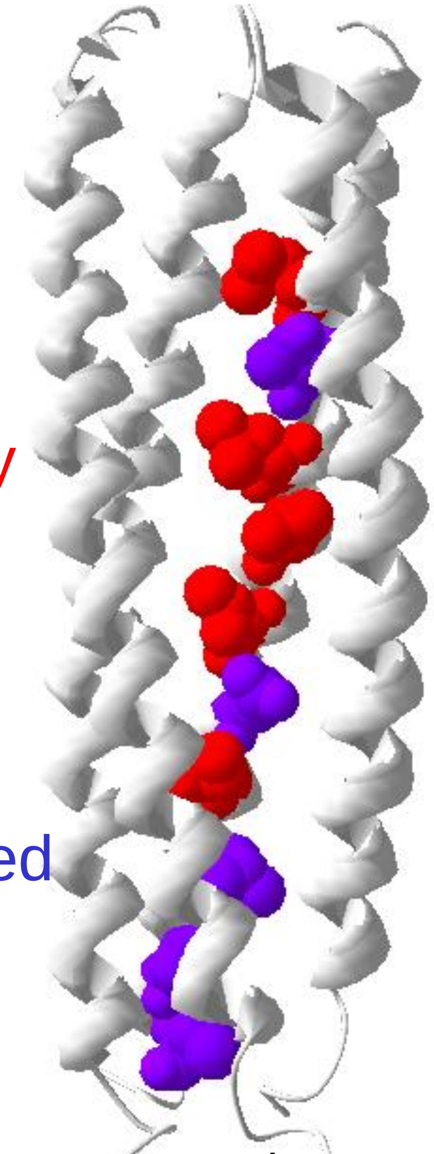
COMPcc (black), L37A (red), T40A (grey), L44A (orange), V47A (yellow), L51A (pink), Q54A (purple), I58A (blue), L61A (green), V65A (light blue), and S68A (light green).

Influence of mutations on stability

Protein	(-VitD) Tm (°C)	(+VitD) Tm (°C)	ΔTm (°C)
wt	44.8	50.9	6.2
L37A	ND	ND	ND
T40A	61.4	62.8	1.4
L44A	ND	ND	ND
V47A	ND	ND	ND
L51A	ND	ND	ND
Q54A	80.3	82.1	1.8
I58A	39.7	39.7	0
L61A	65.3	66	0.7
V65A	86.2	79.7	-6.5
S68A	77	77	0

Stability loss

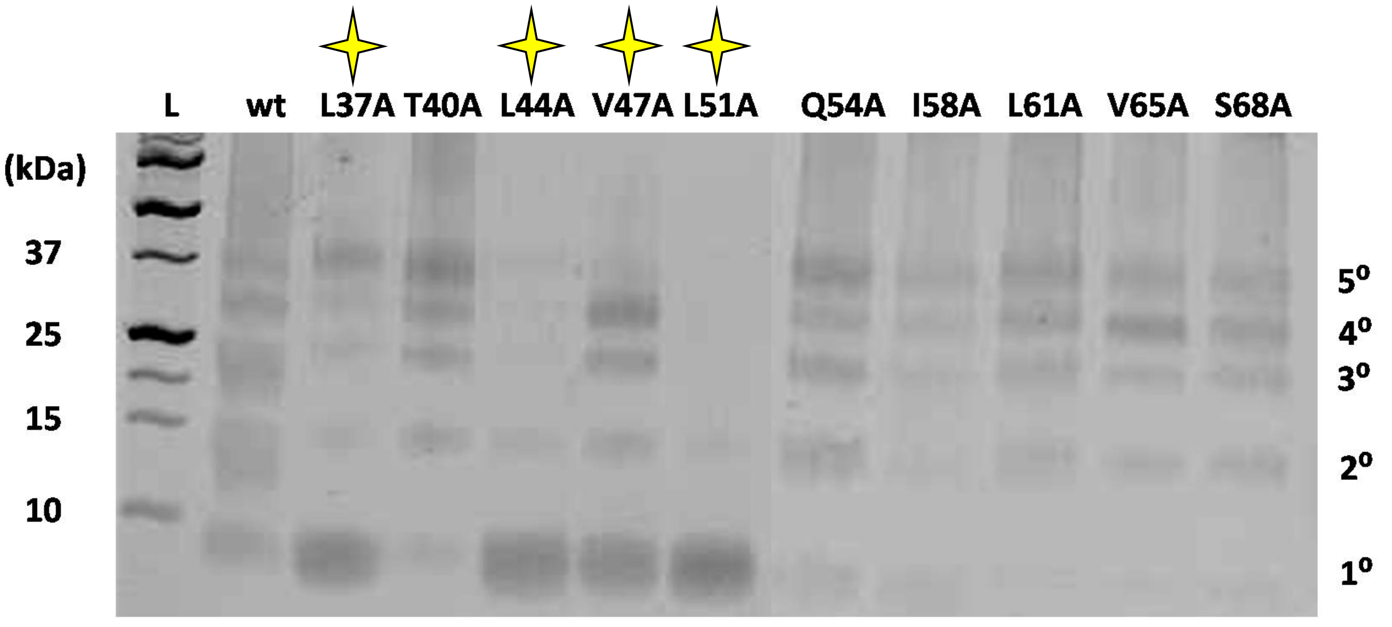
Enhanced stability



- Variants L37A, L44A, V47A and L51A exhibit a complete loss in structure and stability.

S. Gunasekar, M. Asnani

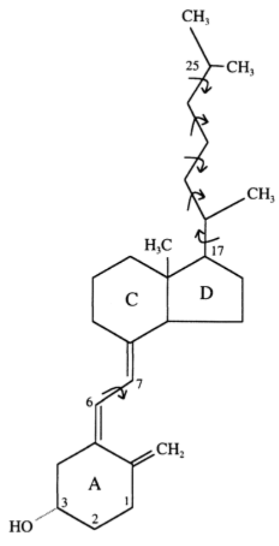
Influence of mutations on oligomerization



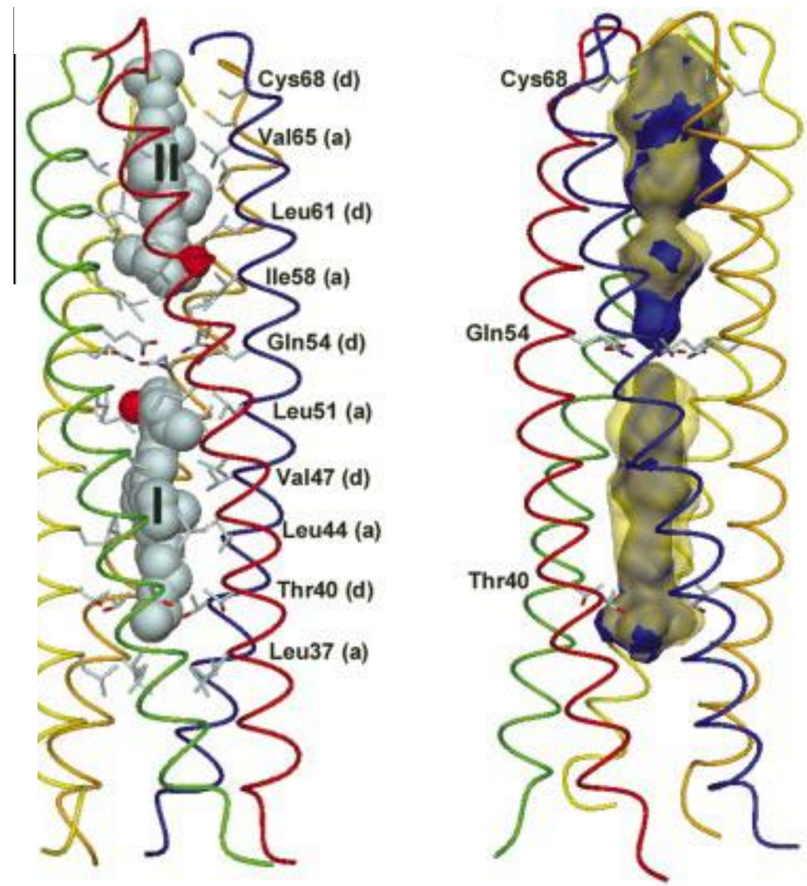
- Variants L37A, L44A, V47A and L51A are unable to form pentamers and are mostly monomeric.

Cartilage oligomeric matrix protein coiled coil (COMPcc)

- Comprised of homopentamer of coiled coils
- Hydrophobic pore 7.3 nm long and 0.2-0.6 nm diameter
- Binds the hormone 1,25-dihydroxy (vitamin D3)

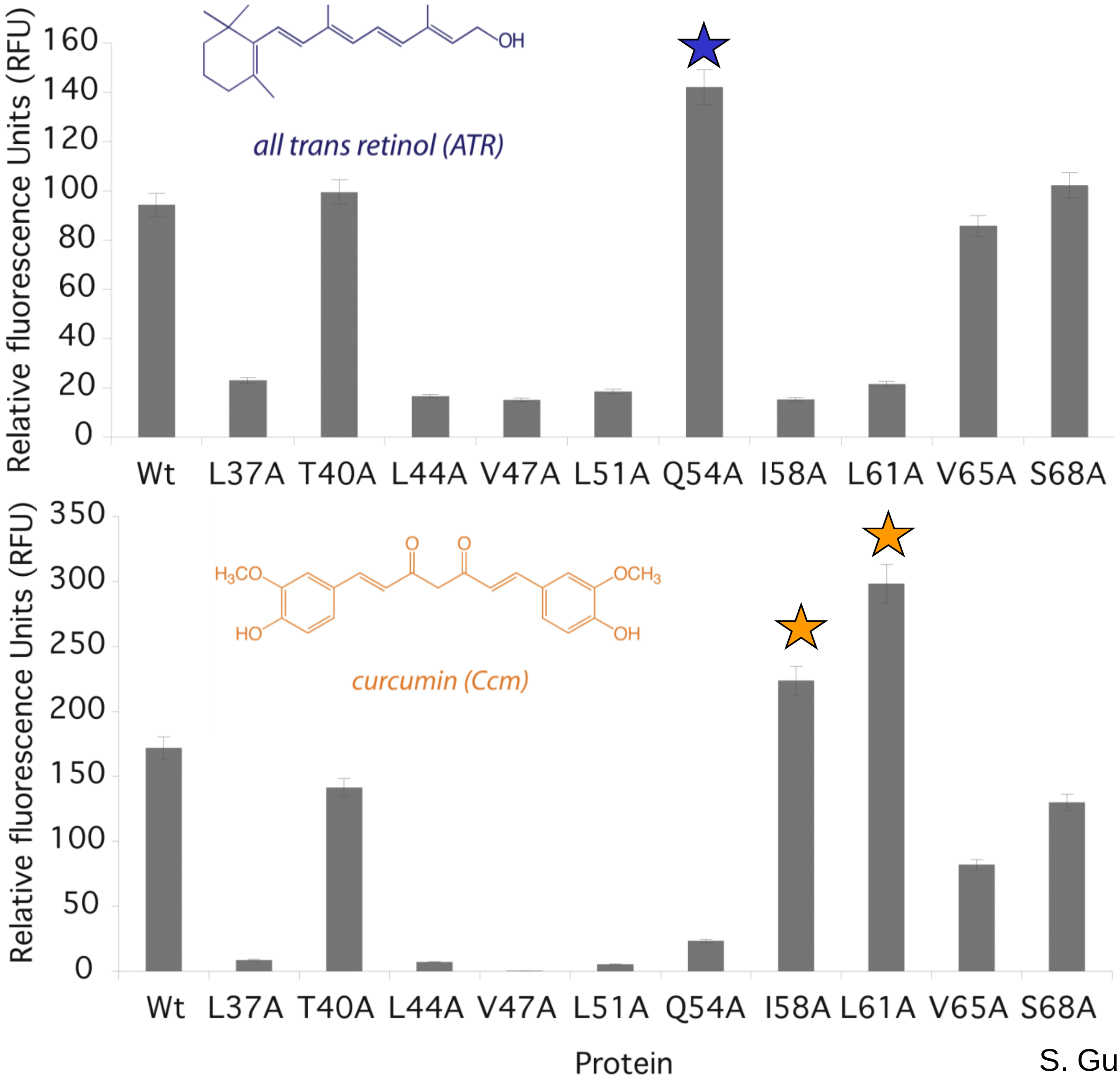


Vitamin D3



Ozbeck, S., Engel, J., Stetefeld, J. *EMBO J.* **2002, *21*,** 5960.

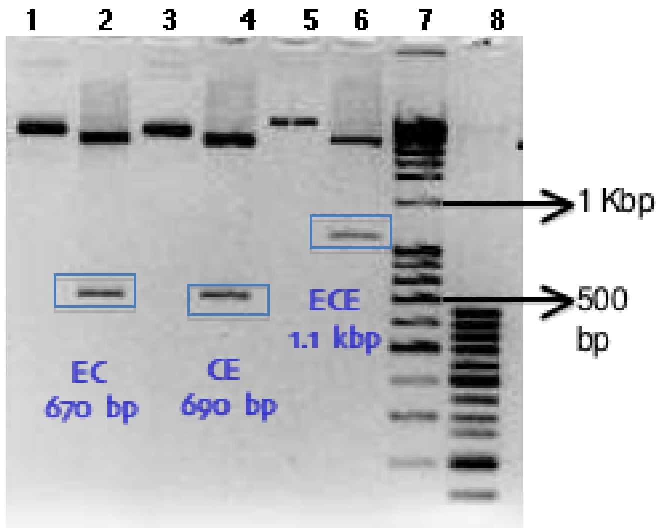
Influence of mutations on ATR and Ccm binding



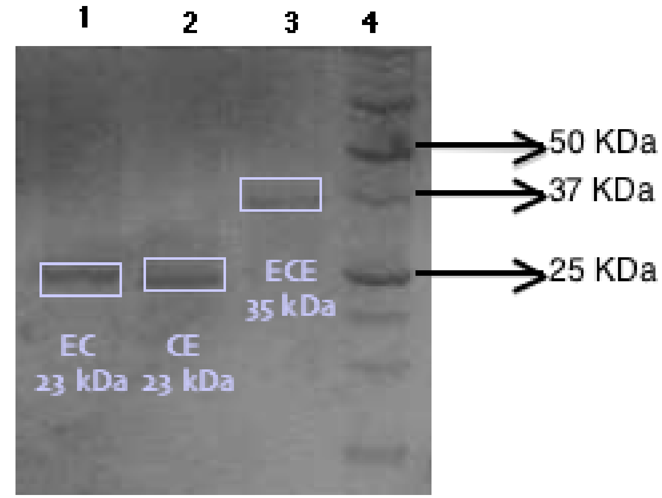
S. Gunasekar, M. Asnani

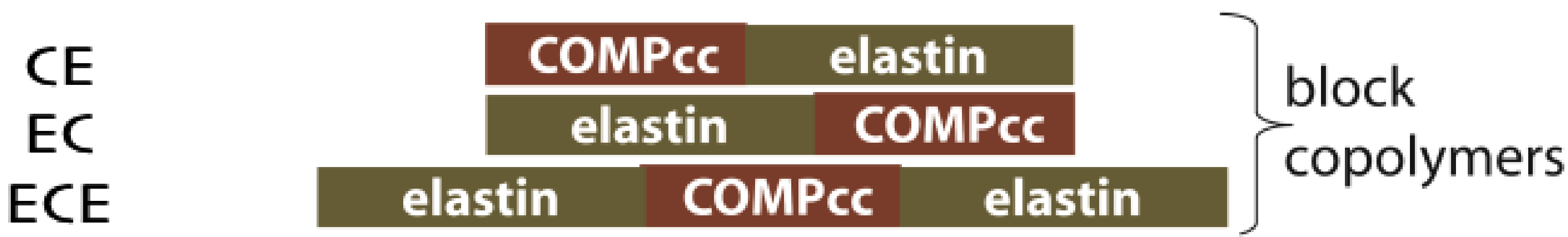
Cloning and Purification

DNA gel



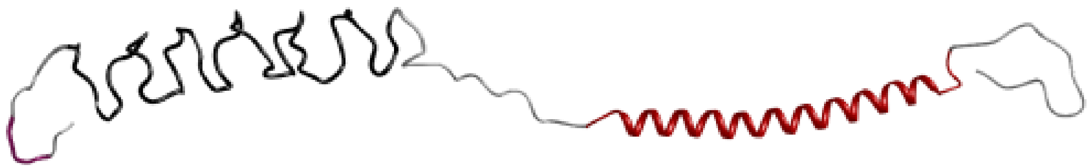
Protein gel





Models of the block polymers with 2 SADS

EC



MRGSH$_6$GSKPIAASA–**E**–LEGSELA(AT)$_6$AACG–**C**–LQA(AT)$_6$AVDLQPS

CE

MRGSH$_6$GSACELA(AT)$_6$AACG–**C**–LQA(AT)$_6$AVDKPIAASA–**E**–LEGSGTGAKLN

ECE

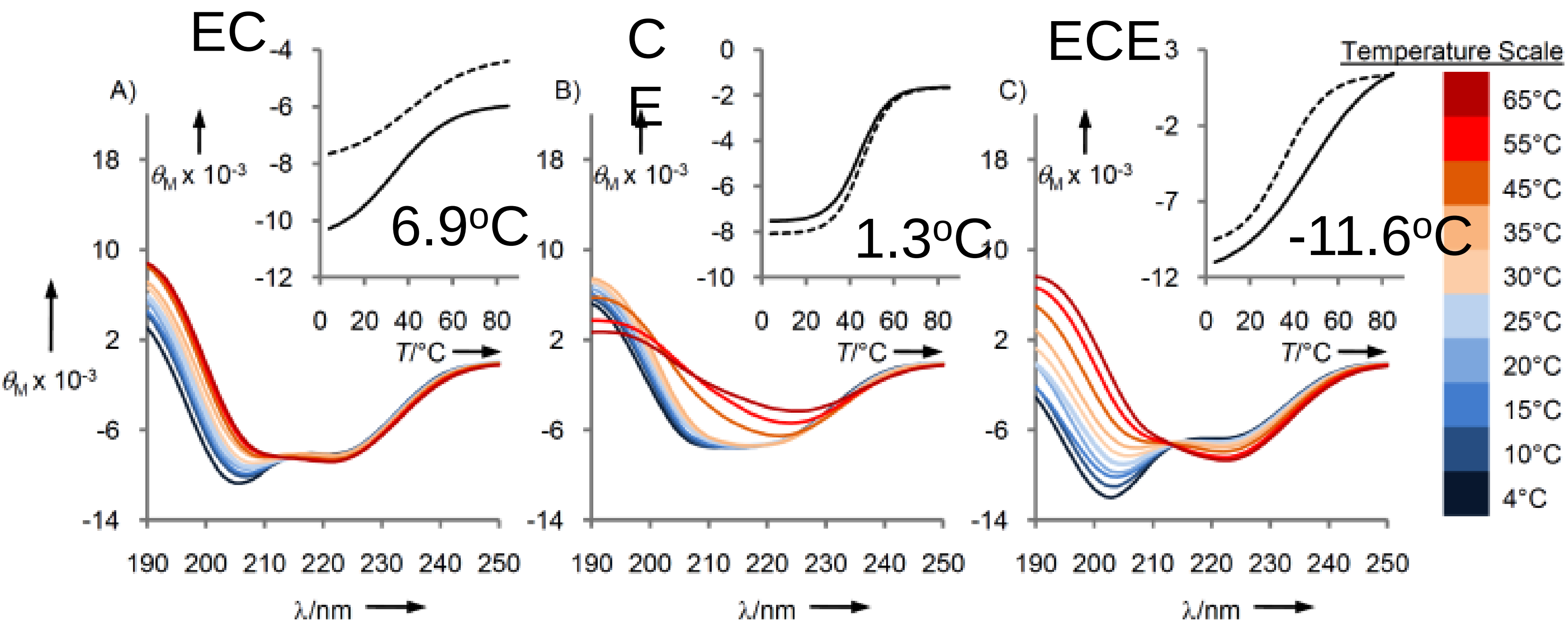
MRGSH$_6$GSKPIAASA–**E**–LEGSELA(AT)$_6$AACG–**C**–LQA(AT)$_6$AVDKPIAASA–**E**–LEGSGTGAKLN

E = [(VPGVG)$_2$VPGFG(VPGVG)$_2$]$_5$VP

C= DLAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDASG

C. Yuvienco

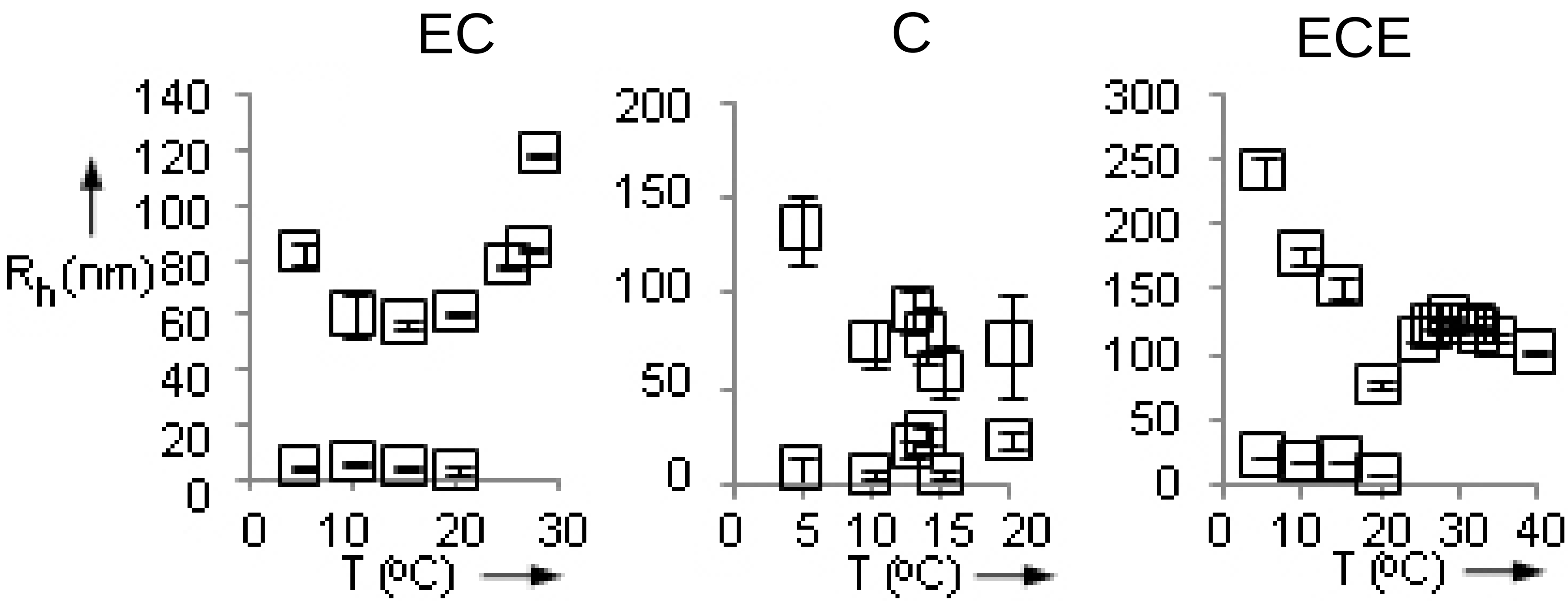
Secondary structure and stability characterization



- The orientation of fusion does make a difference on overall structure of di-blocks
- The number of blocks play an important role in overall conformation and temperature dependent behavior of block polymers
- Influence of vit D on the polymer structure and assembly is dependent on block orientation and composition

J. Haghpanah, C. Yuvienco

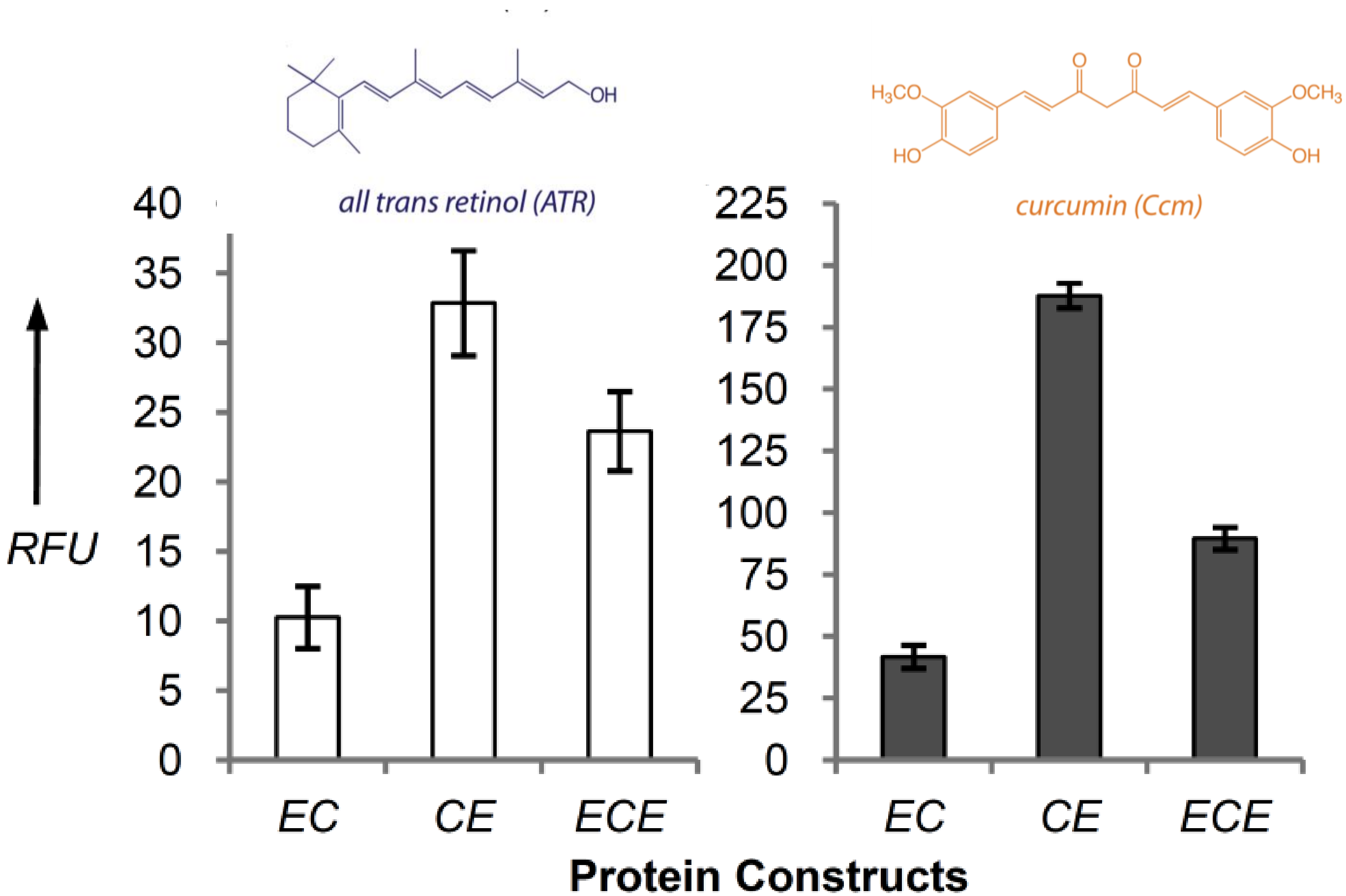
Supramolecular assembly analysis via DLS



- EC: 2 modes where after T_t aggregate size increase; CE: more than 2 modes, polydisperse; ECE: 2 modes where after T_t, size stabilizes to 125 nm; SALS shows EC and CE form micron-sized aggregates
- Orientation and number of blocks affect supramolecular assemblies

D. Civay, M. Muthukumar

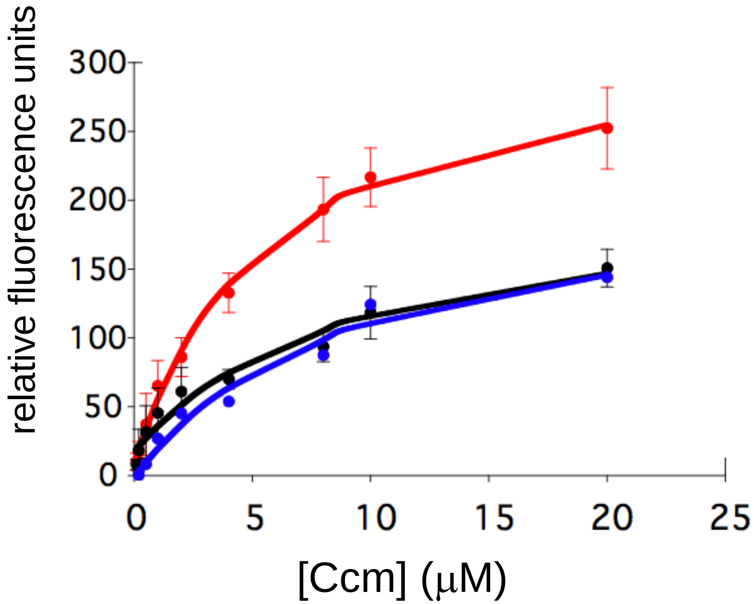
Block Polymer Binding of ATR and Ccm via Fluorescence

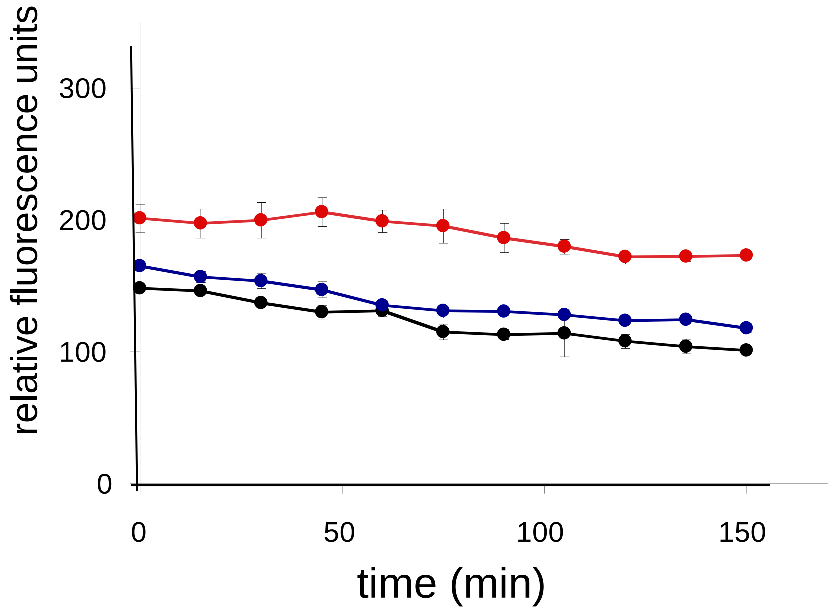


- **CE: binds best to ATR and Ccm indicating importance of N-terminal C domain**
- **ECE and EC: additional C-terminal E domain improves binding**

J. Haghpanah, H. Barra

Binding and Release of Ccm





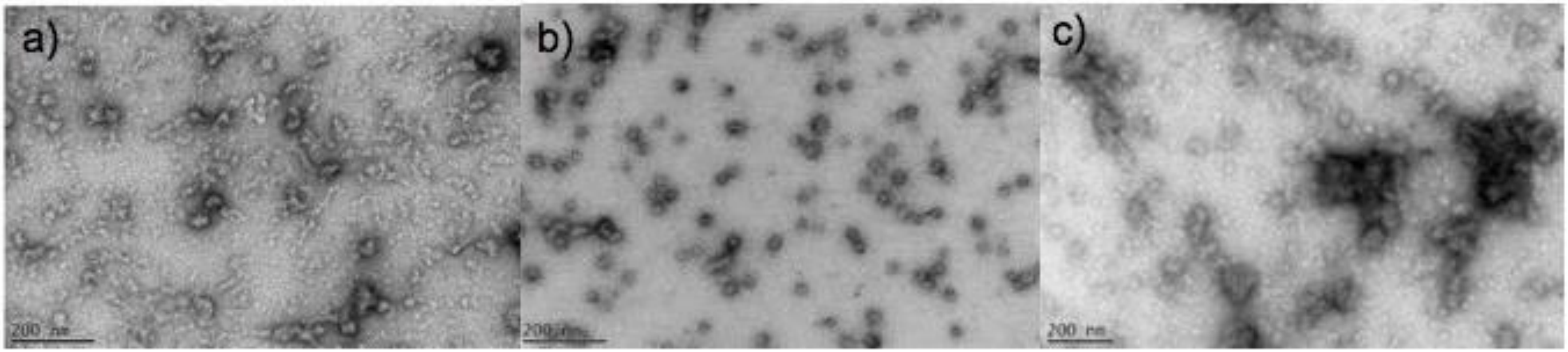
EC	CE	ECE
Kd = 9.26 +/- 1.057 μM	Kd = 5.707 +/- 1.057 μM	Kd = 9.1 +/- 0.077 μM
r^2 = 97%	r^2 = 97%	r^2 = 97%

- **CE exhibits best binding and release abilities relative to both EC and ECE**

J. Haghpanah

TEM Analysis of Block Polymers: Particle-Fiber Swit

EC　　　　C　　　　ECE
　　　　　E

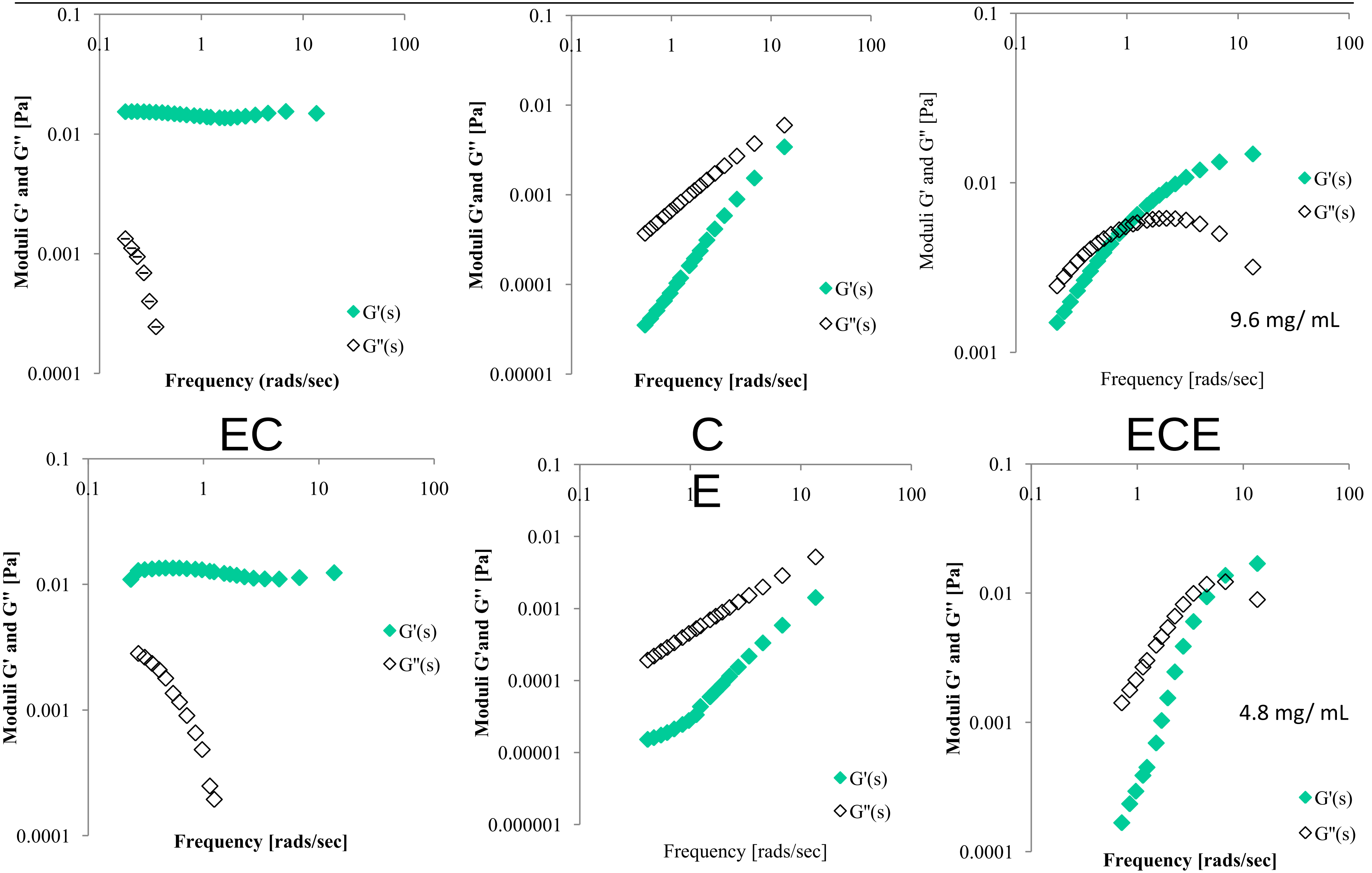


33.8-40.1 nm　　　26.9-29.8.1 nm　　　31.5-39.2 nm

- **EC and ECE look to have similar features with slightly larger sizes when compared to CE, consistent with DLS**
- **While ECE is larger in molecular weight, the article sizes are slightly smaller than EC.**

J. Haghpanah, E. Roth

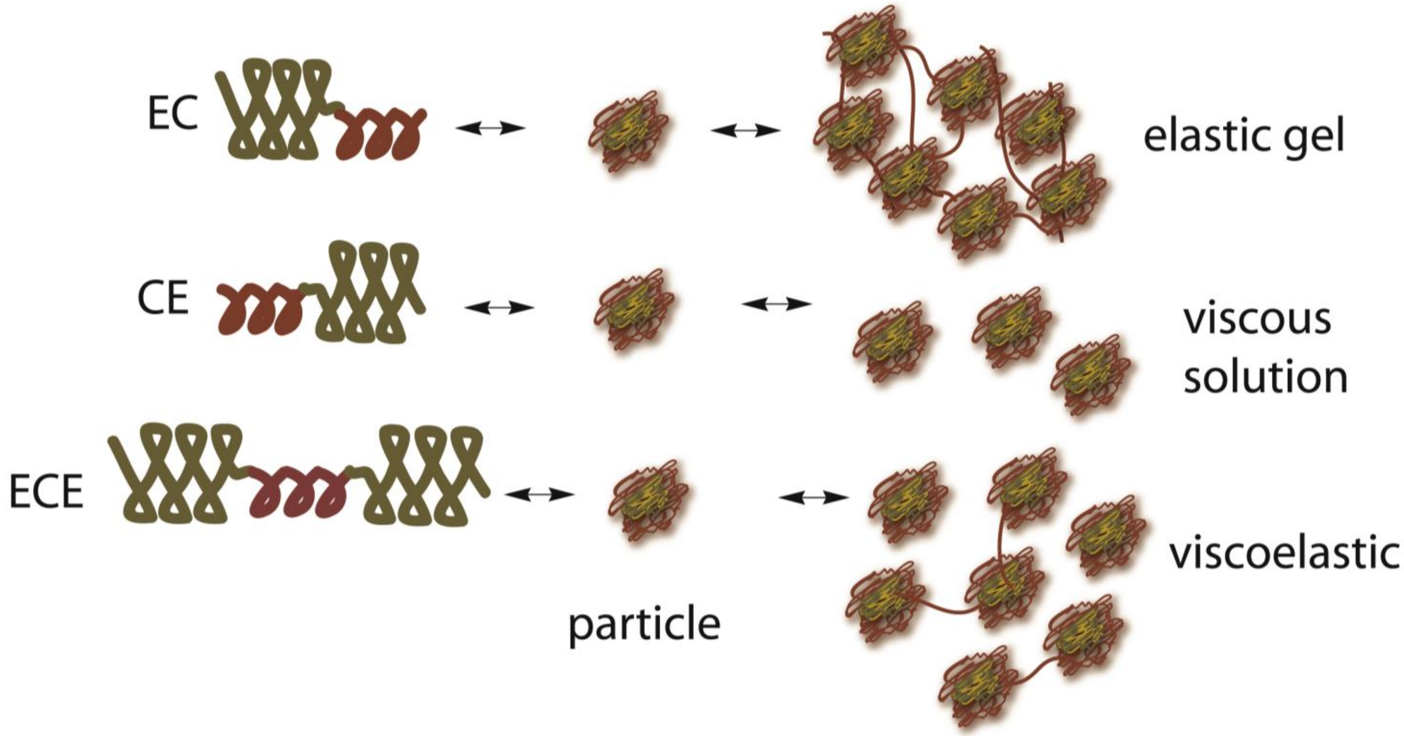
Microrheology of Block Polymers



- **EC: elastic, CE: viscous and ECE: viscoelastic--orientation and block number important**

J. Haghpanah, R. Tu

Supramolecular assembly is dictated by SADs



- **The orientation and number of block influence the supramolecular assembly.**

Acknowledgments

Montclare Lab:

Mukta Asnani
Peter James Baker
Susheel Gunasekar
Jennifer Haghpanah
Wendy Hom
Carlo Yuvienco
Anita Y. Zhu
David Snydacker
Natalya Voloshchuck
Zhiqiang Liu

Funding:

Othmer Institute
Unilever
NSF IUCRC, NSF MRSEC, NSF MRI
CRAW-MROW
AFOSR
Dreyfus Foundation
DOE
ACS/PROGRESS

NYU·poly
POLYTECHNIC INSTITUTE OF NYU

Cast and Crew

SUNY DOWNSTATE Medical Center