# Biomolecular Programming of Discrete Nanomaterials:

## *Enzyme Responsive Polymeric Materials*


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# Biomolecular Programming of Discrete Nanomaterials for Sensors, Templates and Mimics of Natural Nanoscale Assemblies

**Control over morphology on the nanoscale is possible utilizing biomolecules as synthons and stimuli**

## STATUS QUO

**"Smart" Materials –** Should offer routes to autonomous particles capable of responding and adapting to their environment: Sensors, self-healing, remediation, *adaptative concealment* (camouflage), function switching, encoding

**Nanoparticles-** Offer a unique material predisposed for these functions. Current, most predictable strategy is to prepare micelles from small molecule or polymeric amphiphiles

**Stimuli-Responsive materials**

-Usually non-informational

-Can be multi-responsive

## NEW INSIGHTS

**Use biomolecules as polar head groups in brush copolymers for building "smart" particles**

-Morphology is encoded in biomolecular sequence information

**Informational, stimuli-responsive materials**

-Easily multi-input responsive

-Polymer chemistry is now subject to biochemical stimuli – lending superb selectivity and predictability

**Enzymes in Materials Chemistry**

-Explore enzymes as tools in *building complexity* into nanomaterials

## MAIN ACHIEVEMENTS

-Established DNA-nanotechnology as a tool for building and programming polymeric micelles of defined and switchable morphology

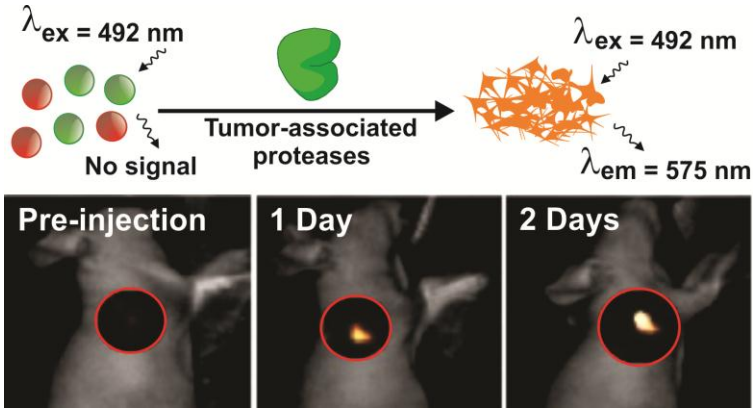
-Established Peptide-Polymer Amphiphiles (PPAs) as tools in the preparation of well-defined, enzyme-responsive materials

-Established FRET-labeled amphiphilic copolymers as a means for the determination of structural and stability parameters of polymeric micelles

-Established that biopolymer-polymer amphiphiles generate predictable, reversibly switchable nanoparticles

-Established enzyme-directed assembly of probes in very complex milieu, including in vivo

-Established concepts of resistant substrates and activated substrates through polymer and/or particle morphology




## QUANTITATIVE IMPACT

**IMPACT ACHIEVED**

*Demonstrated DNA- and Peptides as programming tools in the preparation of shape-shifting micelles*.

Published 3 articles on this subject in 2012, plus 3 currently in review and a patent.

Demonstrated unusual morphology-dependent resistance

*Demonstrated that DNA- and Peptide-brush polymer nanoparticles form robust, semiautonomous systems capable of performing in biological milieu*

## END-OF-PHASE GOAL

**RESEARCH GOALS**

To enable the predictable synthesis of discrete organic nanoparticles – to achieve a level of control known only in synthetic organic chemistry and biology, on the nanoscale.

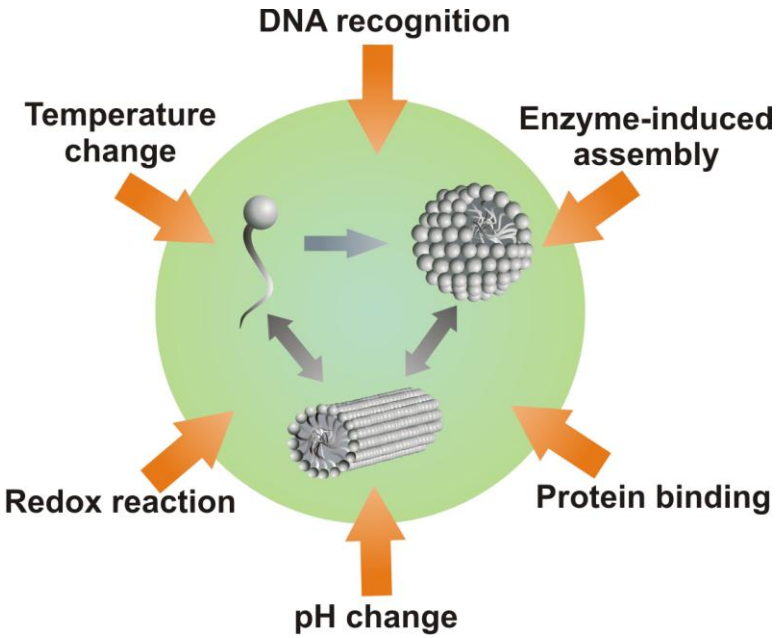
To enable the development of highly dynamic materials capable of stimuli-induced changes in morphology and function.

To enable autonomous, or remote-control over structures with the physical and replicative properties of biomolecular nanomaterials – e.g. virus capsids

# Program Goals

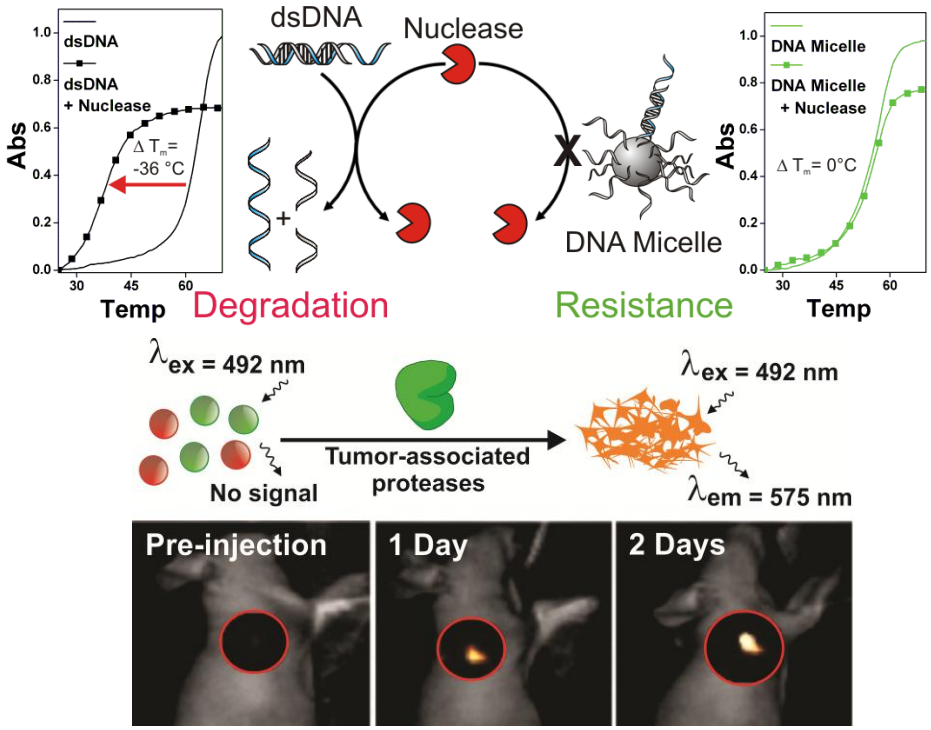
***Developing Adaptable, Autonomous Chemical Systems***

**Goal 1: Developing the concept of biomolecule programming of discrete polymeric nanomaterials**




"Enzyme-directed assembly and manipulation of organic nanomaterials"
Hahn, Gianneschi; *Chem. Commun.* **2011**, 47, 11814-11821

**Goal 2: To explore morphology switchable materials in biochemical systems**

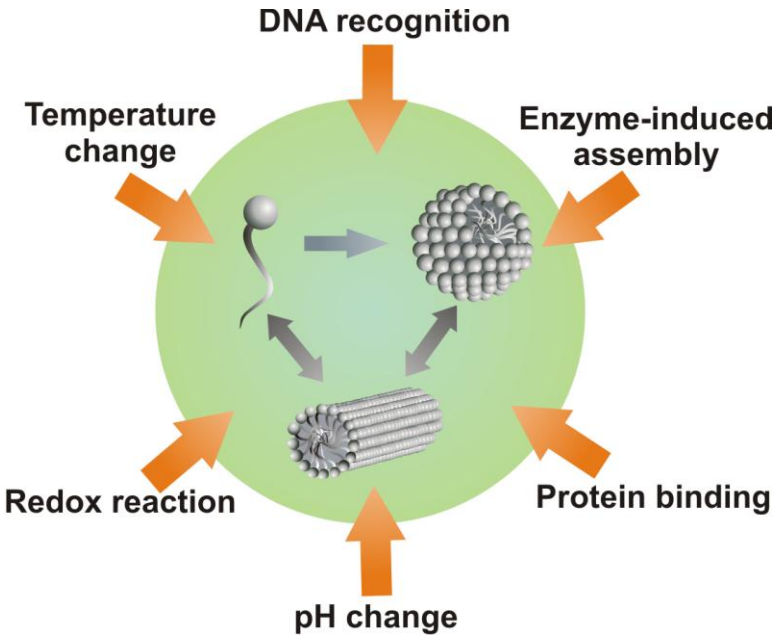


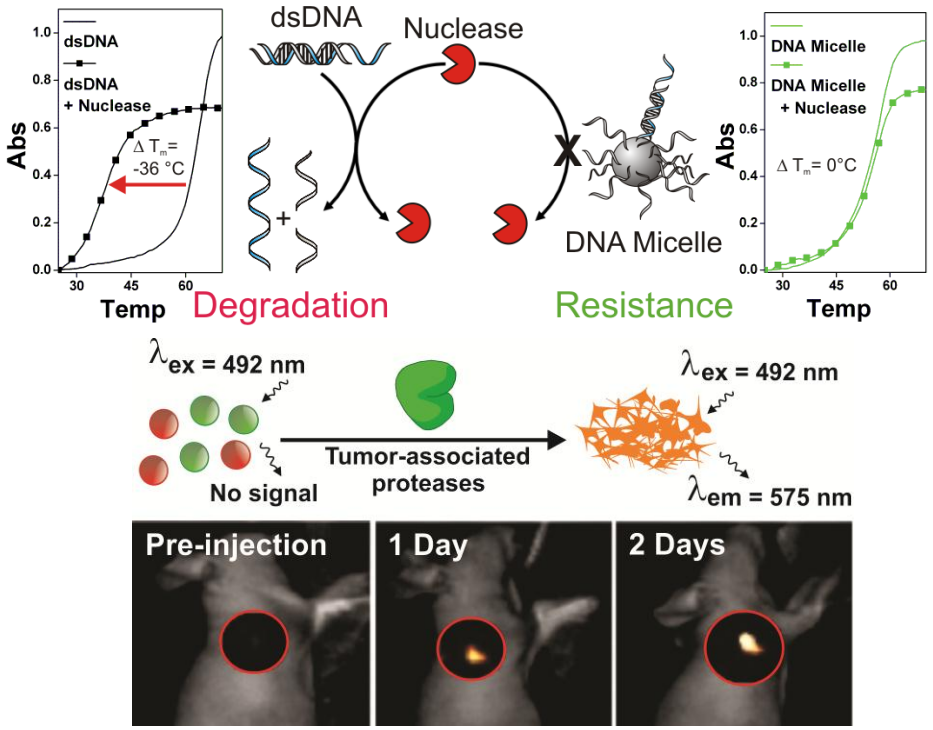

"Biological stimuli and biomolecules in the assembly and manipulation of nanoscale particles"
Randolph, Chien, Gianneschi; *Chem. Sci.* **2012**, 3, 1363-1380

# Program Goals

## *Developing Adaptable, Autonomous Chemical Systems*

The ability to utilize morphology switches in detection of biochemical events will be a special focus of this work and will involve a study of various interactions with living systems. FRET-based systems will be utilized for detecting morphology change and/or enzyme-triggering. Motivation for exploring the fundamental aspects and basic limitations of these materials in a variety of environments comes from the possibility for developing in vivo sensors and stimuli-responsive systems for detection and response

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# Progress Towards Program Goals

**Achieved December 2011-present**

- Demonstrated DNA and peptides can be used to control and program the assembly and morphology of nanoparticles (routinely achieved in natural materials) – beyond non-informational stimuli, and static systems
- Demonstrated that this approach is robust – Increases stability of biomolecules. Approach works in complex biological milieu (blood stream/organs of living organism) allowing “remote-control”, and pre-programmed autonomous systems
- Demonstrated enzyme-driven manipulation of nanomaterials – Largely the domain of natural systems.
- Demonstrated substrate scope with regards to polymerization of peptides, and ultimately seek to establish a novel approach to protecting peptides as brush polymers – a new way of maintaining bioavailability but preventing degradation

**Currently pursuing and aim to pursue this year**

- Currently investigating how peptides and nucleic acids can be arrayed on, and as parts of nanoparticles for responsiveness, programmability or resistance to their environments
- Aim to gain tight control over phase transitions governed by enzymatic processes – To date a limited number of transitions have been satisfactorily demonstrated
- Aim to explore a broader range of enzymatic systems including transpeptidases and other systems that are being pursued currently in our labs
- Aim to demonstrate the enzyme-directed assembly of nanomaterials from simple, low molecular weight polymer subunits – i.e. guide particle formation utilizing enzymatic “tools”
- Aim to work on approaches for propagating nanoscale responses studied so far to longer length scales
- Aim to work on DNA-based nanoparticle systems for organizing protein and/or nucleic acids for function in cell cytosol

# Programming Nanomaterials with Biomolecules

**This program seeks to tackle key problems inherent to dynamic soft materials :**

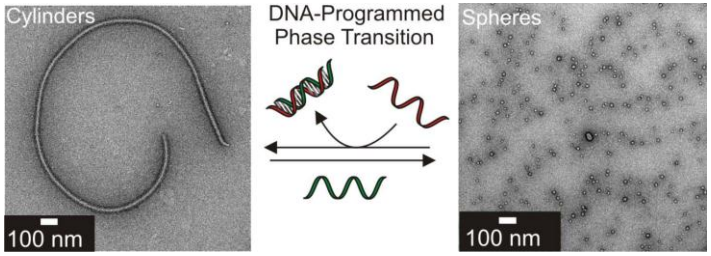
1) Synthetic problems

1) Characterization problems

1) Chemical systems problems (molecular ecosystems)

## Nucleic acid programmed materials

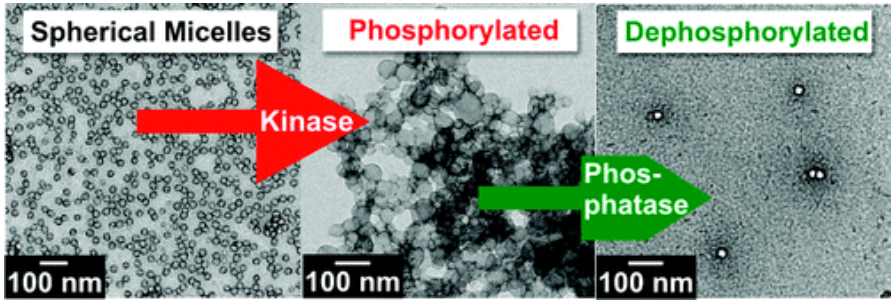
*DNA-Polymer Amphiphiles - DPAs*




Chien, et alia. *Angew. Chem.* **2010**
Thompson, et alia. *Nano Lett.* **2010**
Chien, et alia. *Chem. Comm.* **2011**
Chien, et alia. *Small*, **2011**
Chien, et alia, *Submitted,* **2012**

## Peptide/enzyme programmed materials

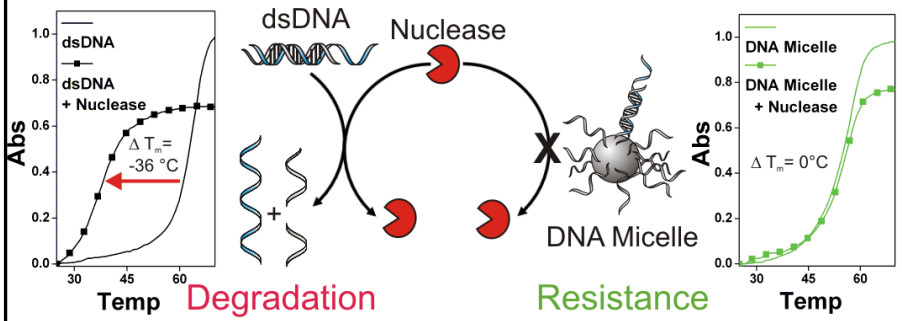
*Peptide-Polymer Amphiphiles - PPAs*




Ku, et alia. *J. Am. Chem. Soc.* **2011**
Hahn, et alia. *Chem. Comm.* **2011**
Randolph, et alia. *Chem. Sci.,* **2012**
Chien, et alia. *Chem. Sci.,* **2012**
Chien, et alia. *Submitted,* **2012**

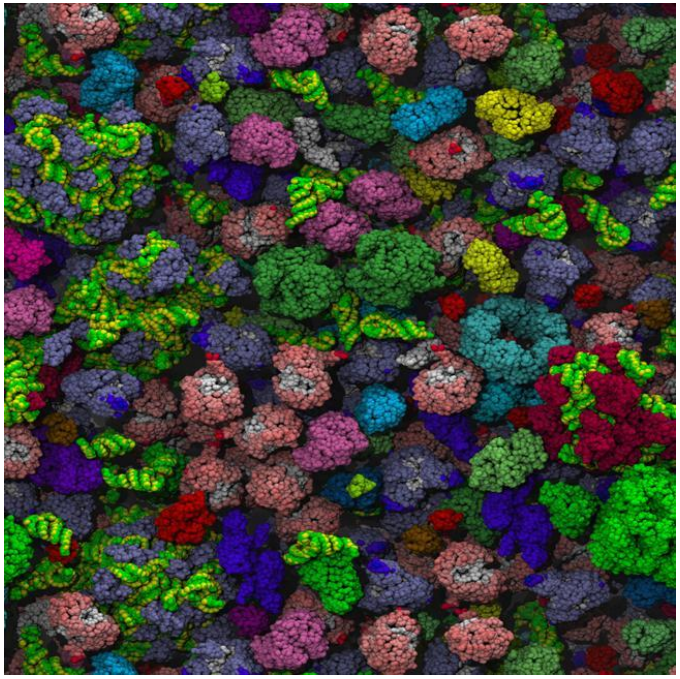
## Resistant biomolecules, stabilized as hybrid polymers and polymeric nanostructures

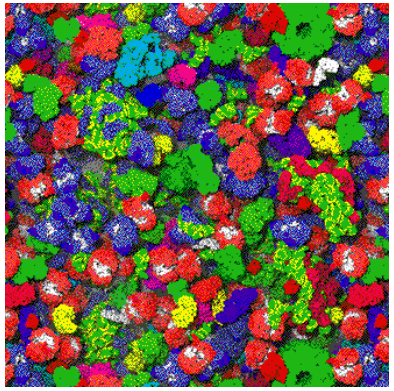
*Applications in therapeutics and advanced materials*



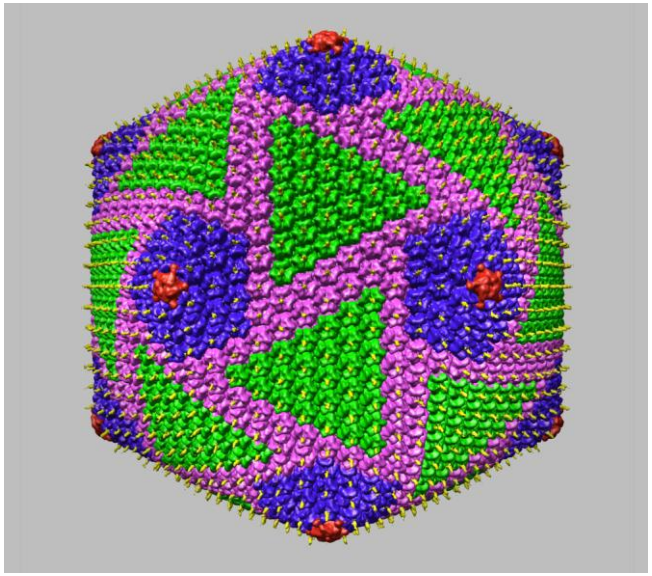

Rush, et alia. *Submitted,* **2012** *(nucleic acid based materials)*
Hahn, et alia. *In preparation (peptide-based materials)*

# Complex Materials, in Complex Environments: A Challenge to Biochemistry and Synthetic Chemistry





The crowded cytosol modeled at 275 g/L of proteins (20 μs)
McGuffee, S. R.; Elcock, A. H. *PLoS Comput. Biol.* *Doi:10.1371/journal.pcbi.1000694,* **2010**



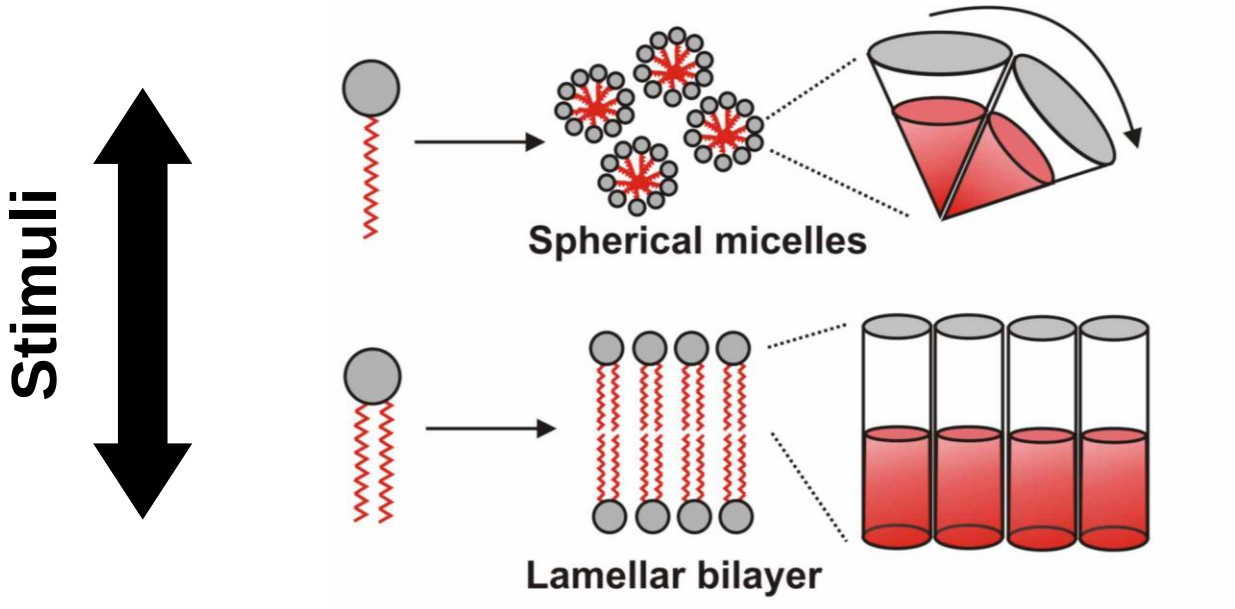
Chilo Iridescent Virus (CIV) – Baker, T.S. cryoEM 3D image reconstruction

Crowding in folding and stability: Gruebele, Pielak, Gierasch

**Can we learn how to stabilize biomolecules in non-natural environments?**
*Learn how to use biomolecules as structural elements within synthetic materials. In particular, where the biomolecule programs the morphology of the material, and the synthetic moeties stabilize the structure and guide the chemistry*

**Can we learn how to self-assemble synthetic nanoscale, soft materials in complex biological environments?**
*Learn how to mediate selective interactions in highly competitive environments by utilizing biomolecules coupled with synthetic polymers*

# Developing Materials that Change Morphology in Response to Stimuli

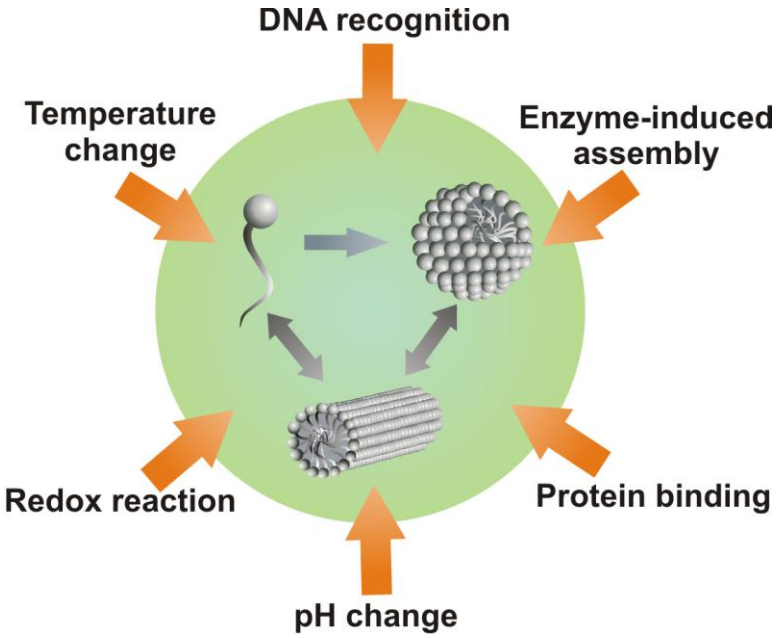


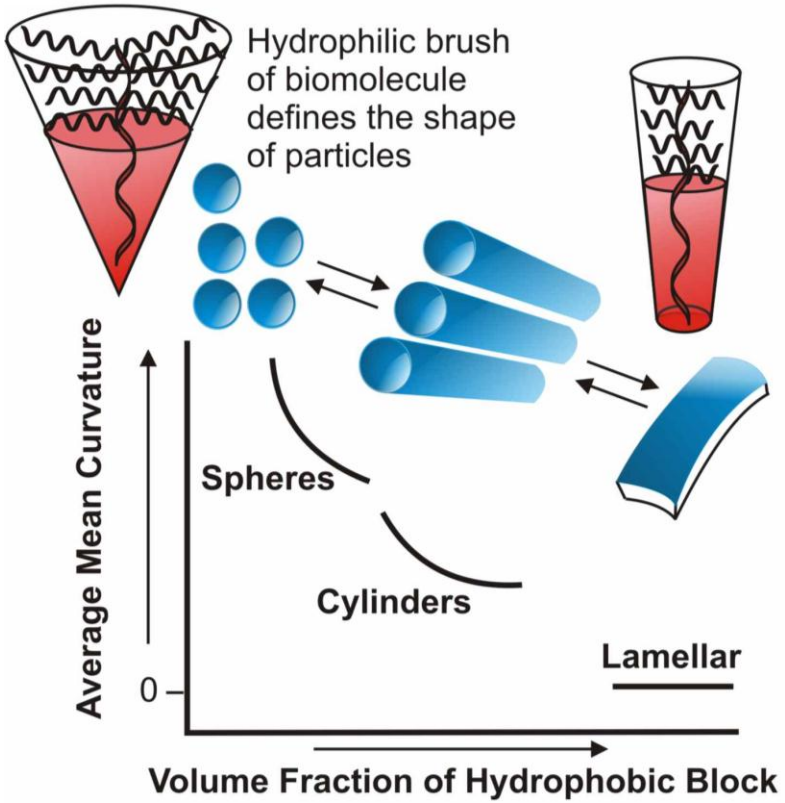

Jain, S. & Bates, F. S. *Science* **300**, 460-464 (2003)

Israelachvilli, J. N., Mitchell, D. J., & Ninham, B. W. *J. Chem. Soc., Faraday Trans. 2: Mol. Chem. Phys.* **72**, 1525-1568 (1976)

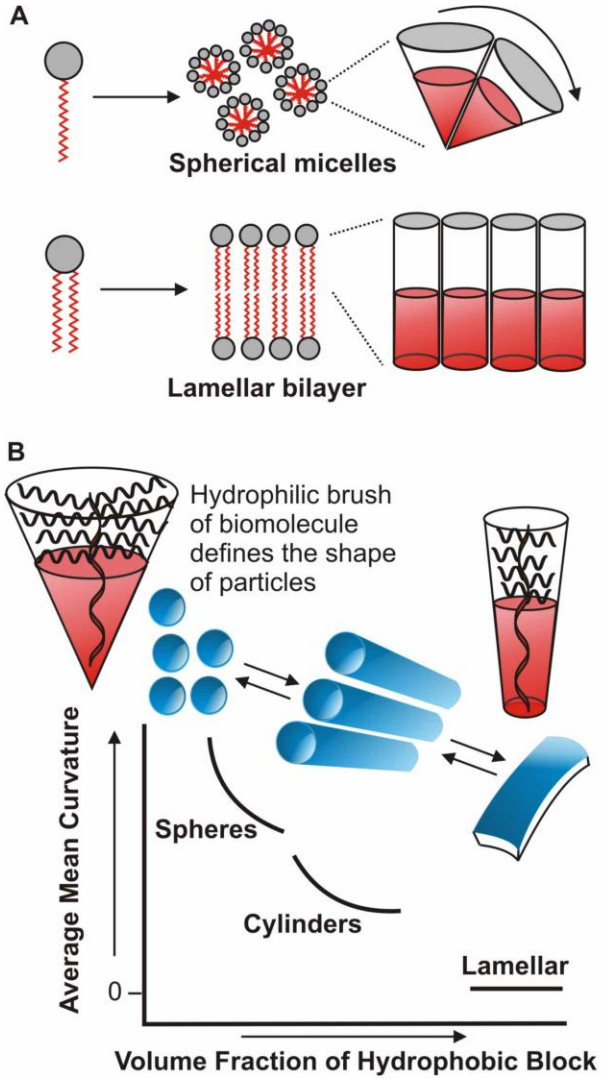
Wang, Y., Xu, H., & Zhang, X. *Adv. Mater.* **21**, (2009)

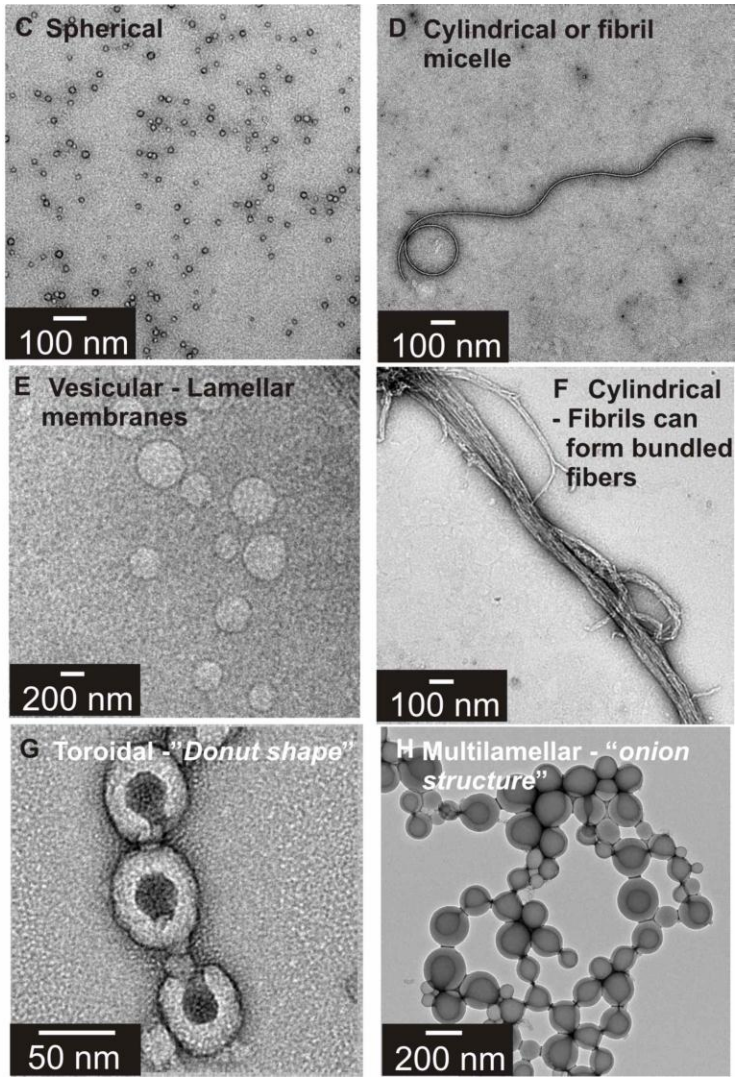
# Programmable Phase Transitions: Nucleic acids, peptides and an array of enzymes



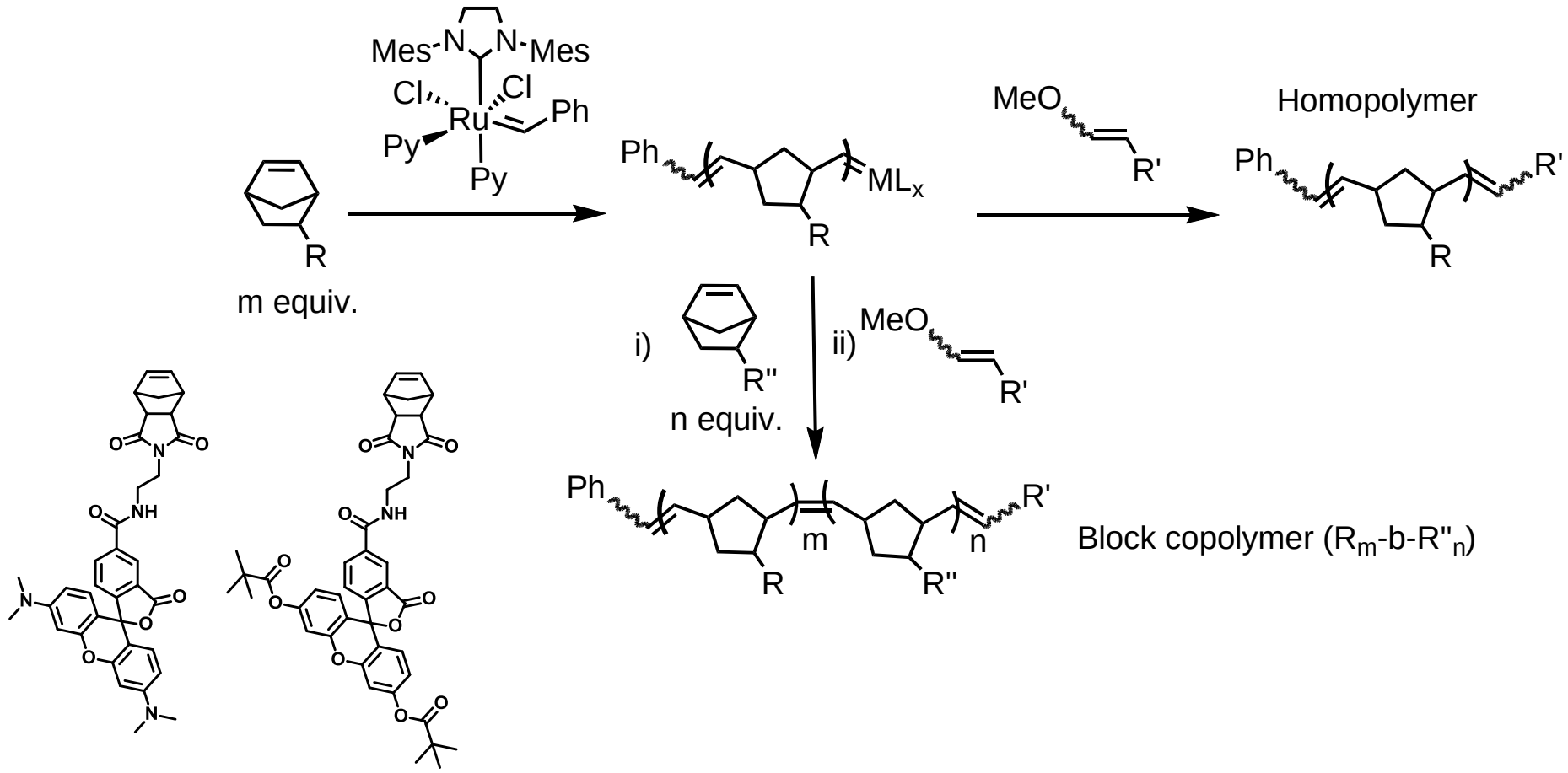



# Examples from our Work of Accessible Phases Utilizing DNA-Programmed Amphiphiles (DPAs)



# Well-Defined Polymers, High Functional Group Tolerance + End-labeling of Termini

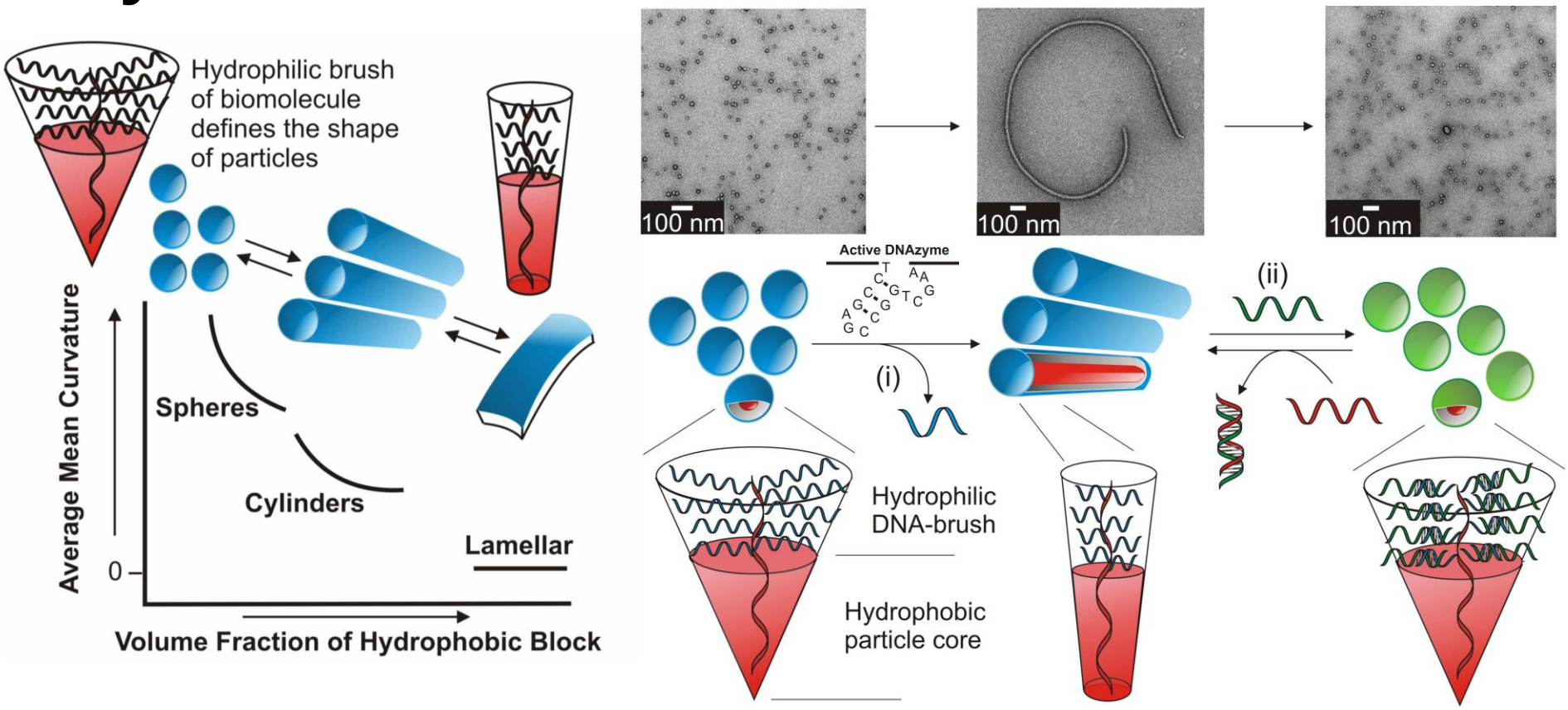




D. Smith, E. B. Pentzer, S. T. Nguyen, *Polym. Rev.* **2007**, *47*, 419.

Y. Xia, B. D. Olsen, J. A. Kornfield, R. H. Grubbs, *J. Am. Chem. Soc.* **2009**, *131*, 18525.

# Programmable Phase Transitions: Nucleic acids, peptides and an array of enzymes




Chien, M.; Rush, A.M.; Thompson, M.P.; Gianneschi, NC. Angew. Chem. Int. Ed., **2010**, *49*, 5076-5080
*Work in progress to demonstrate these responses in biological fluids

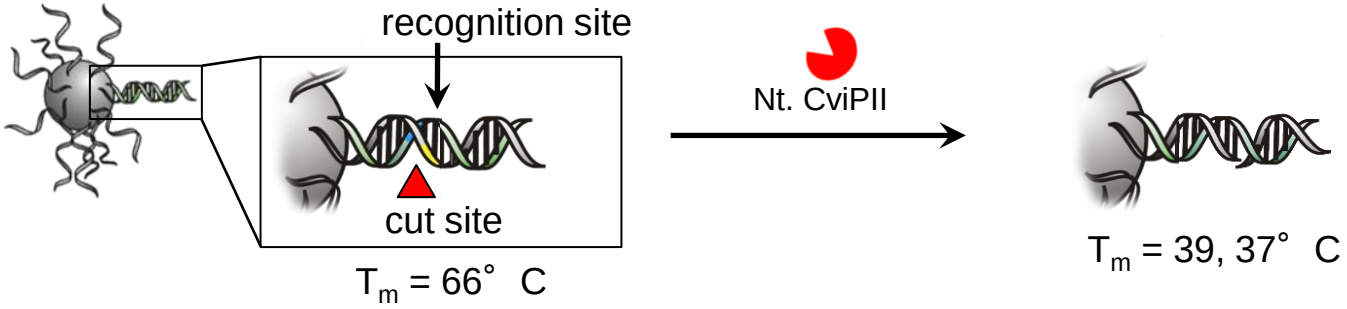
**DNA as the polar head group in block copolymer micelles**
Li, Z., Zhang, Y., Fullhart, P., & Mirkin, C. A. *Nano Lett.* **2004**, *4* 1055-1058
Alemdaroglu, F. E. & Herrmann, A. *Org. Biomol. Chem.* **2007**, *5,* 1311-1320 (*Review*)

# Question of Resistance: Packaging for Protection from Endonucleases and Exonucleases

## Nicking Endonucleases

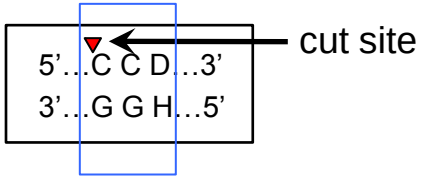
**Normal ssDNA substrates:**
*Nicking occurs only on one strand of a double-stranded DNA helix*

**Particles as substrates:**
*Suppressed activity? Enhanced activity?*



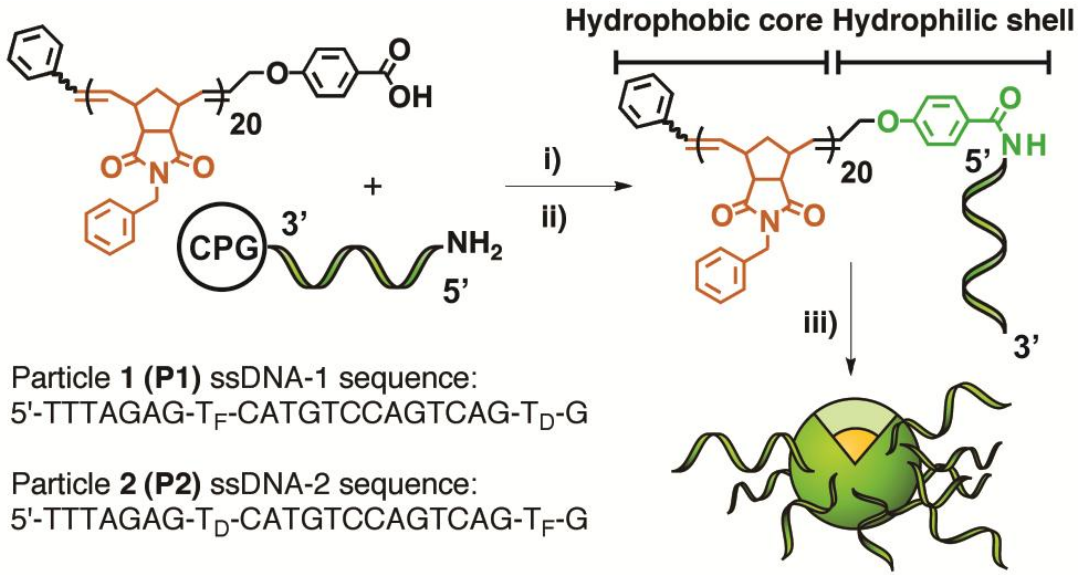

$T_m$ = 66° C

$T_m$ = 39, 37° C



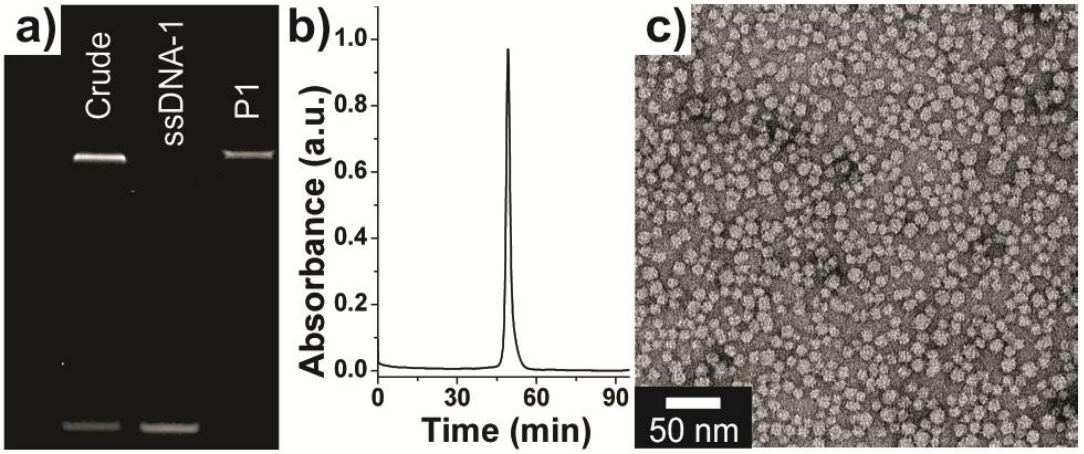

***Anthony Rush, Submitted***

# Synthesis of DNA-Amphiphiles (DPAs) and DPA-nanoparticles




Particle **1 (P1)** ssDNA-1 sequence:
5'-TTTAGAG-$T_F$-CATGTCCAGTCAG-$T_D$-G

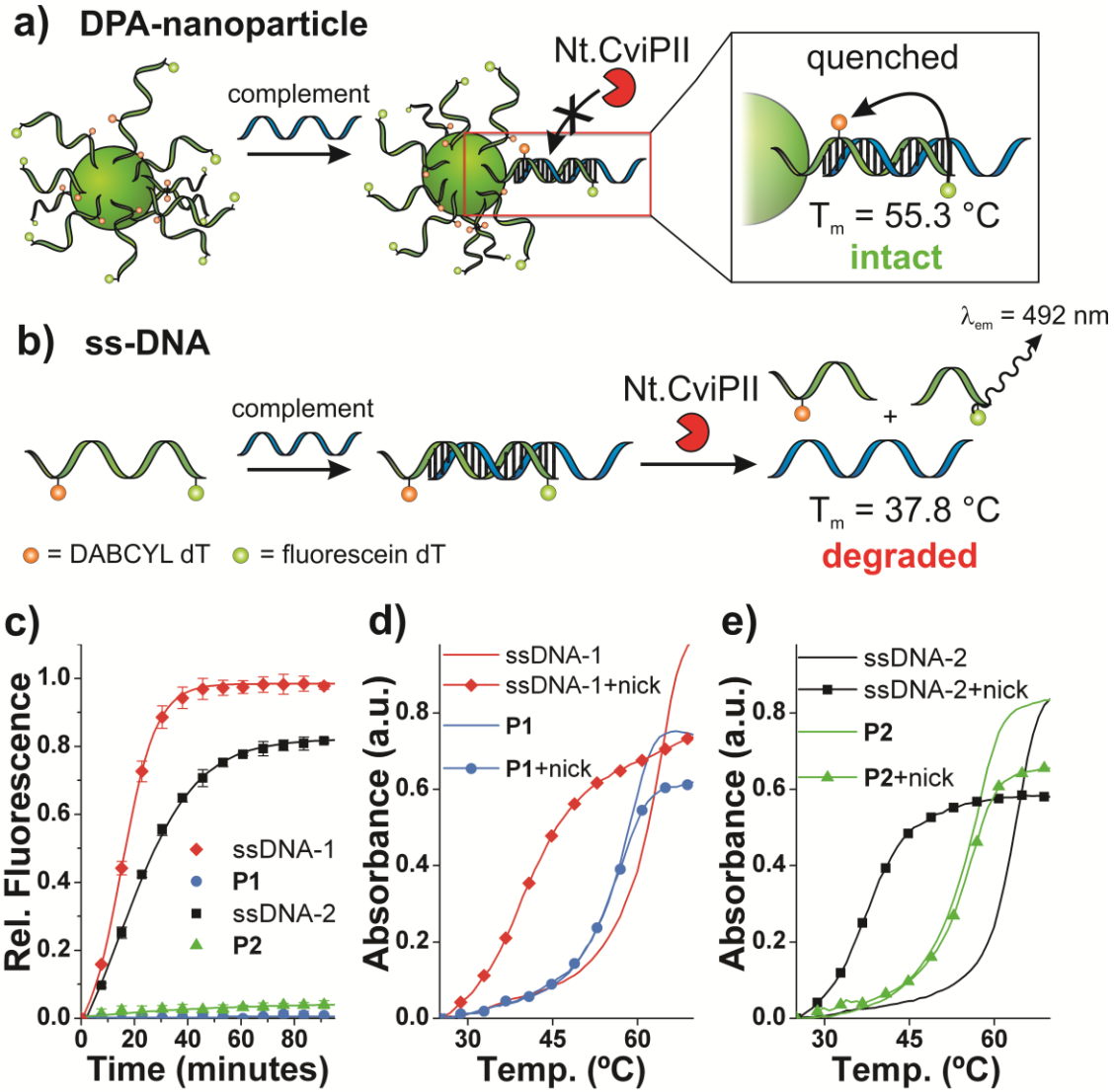
Particle **2 (P2)** ssDNA-2 sequence:
5'-TTTAGAG-$T_D$-CATGTCCAGTCAG-$T_F$-G




- DPAs prepared on solid support – purified from unreacted polymer by washing
- Particles prepared by dialysis – purified from free ssDNA by size-exclusion chromatography (FPLC)
- Purity confirmed by PAGE (a), FPLC (b) and particles analyzed by TEM (c) and DLS (not shown)

***Anthony Rush, Submitted***

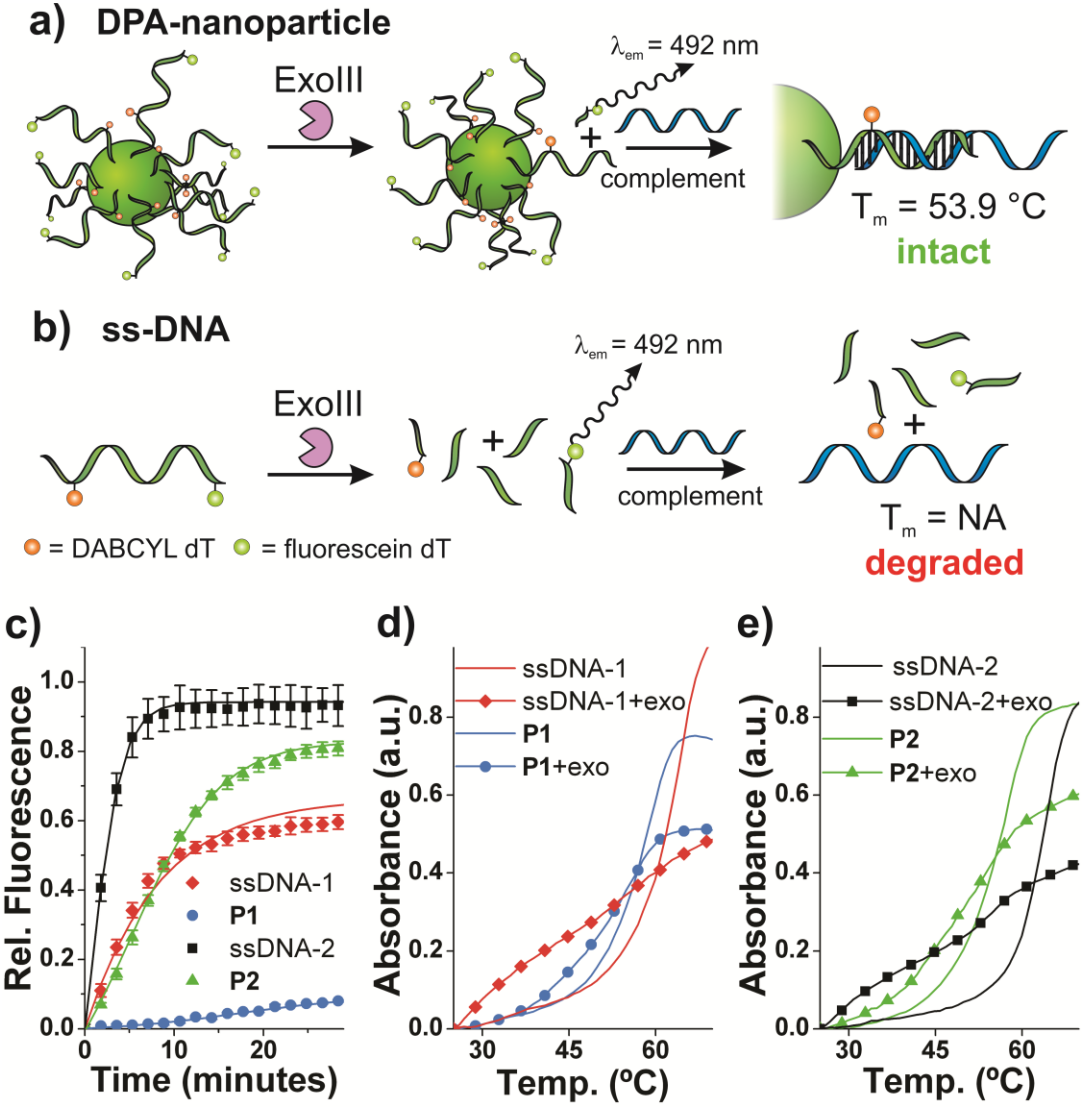
# Nicking Endonuclease Resistance




- Particles exhibit resistance to the nicking endonuclease
- Resistance is observed as a flat signal observed upon excitation of fluorescein
- Resistance is observed as no change in melting temperature of a DNA:DNA duplex in the particle shell

***Anthony Rush, Submitted***

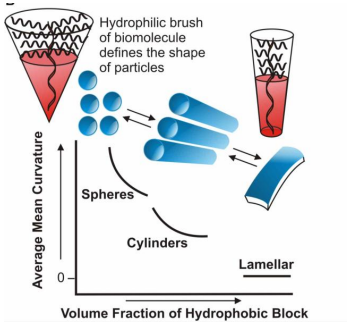
# 3'-Exonuclease Resistance – Shaving Particles with Exo III

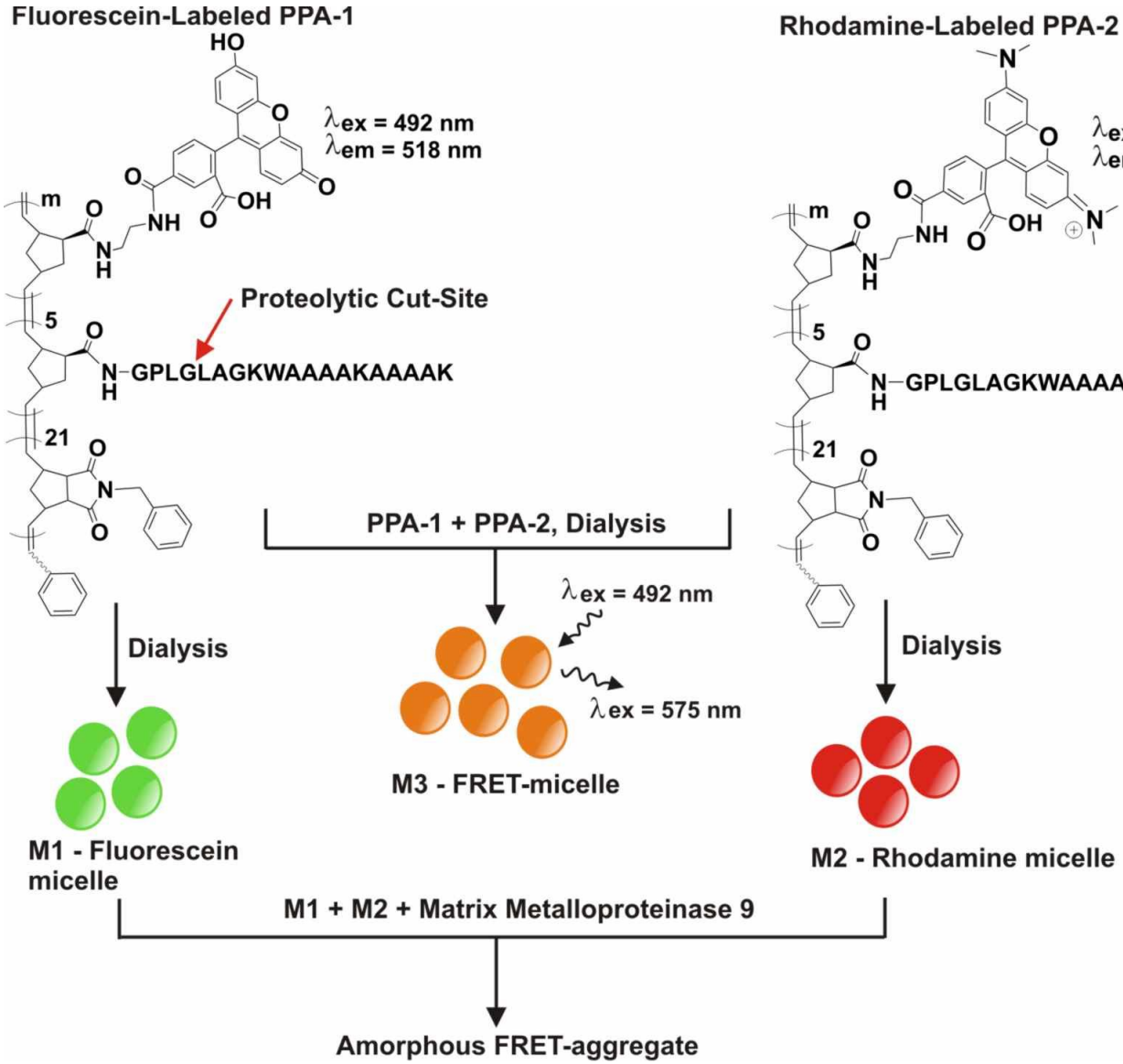



- Particles exhibit resistance to the exonuclease
- Particles are "shaved" by the exonuclease
- Resistance is observed as limited reduction in melting temperature consistent with 2-3 bases missing post-enzyme treatment

***Anthony Rush, Submitted***

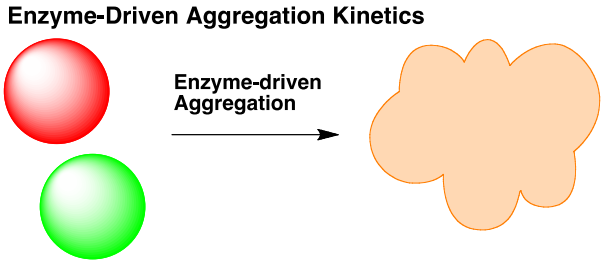
# Fluorogenic Enzyme-Responsive Micellar Nanoparticles – Peptide-Polymer Amphiphiles

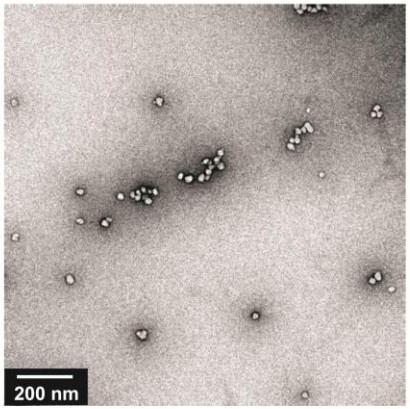


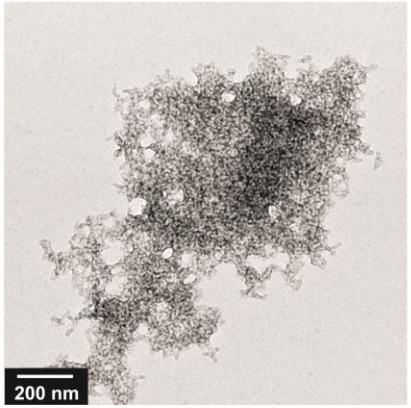



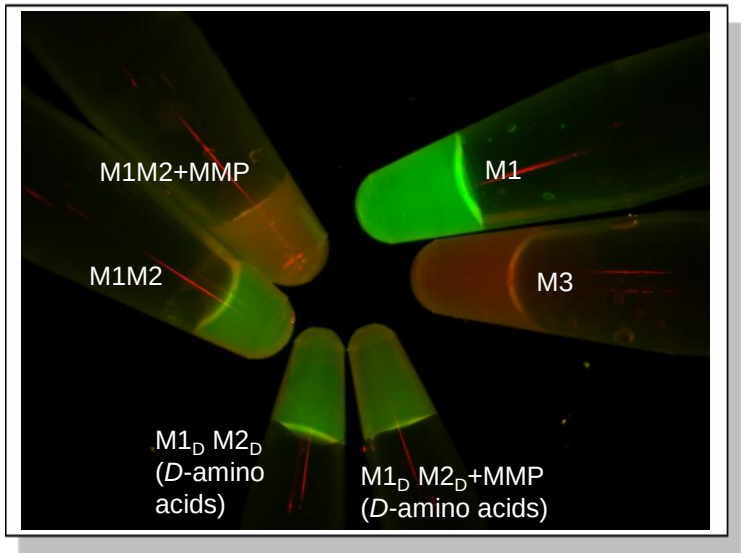

***Miao-Ping Chien,*** *Chem. Sci.* **2012**

# Proteolysis Detected via Particle Morphology Change/ Aggregation



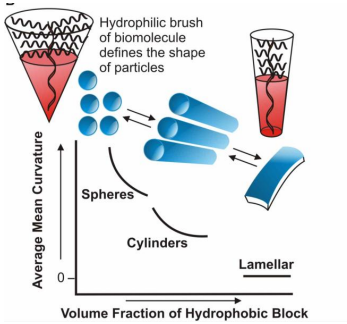



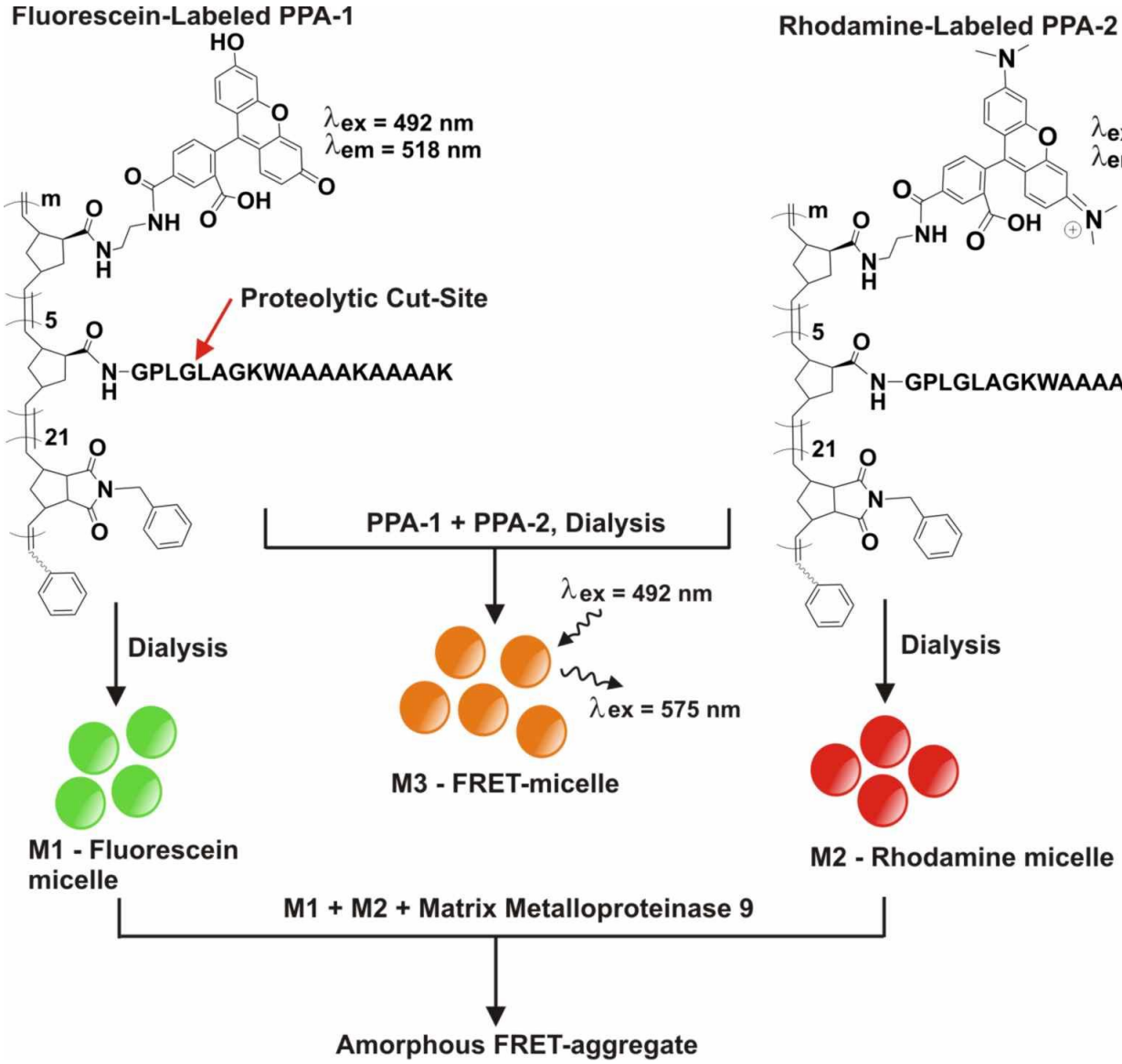




- Spectroscopy reveals FRET signal upon enzyme-driven aggregation reaction
- MMPs are detectable down to 10 pM with 2.5 μM PPA
- PPA concentrations can be as low as 20 nM, and still enable detection of MMP at 10 nM
- System detects cell-excreted MMP-2 and MMP-9 (0.048 nM, and 0.005 nM) WPE1-NA45 cells (MCF-7 as control cells).

# Fluorogenic Enzyme-Responsive Micellar Nanoparticles – Weaknesses of past system

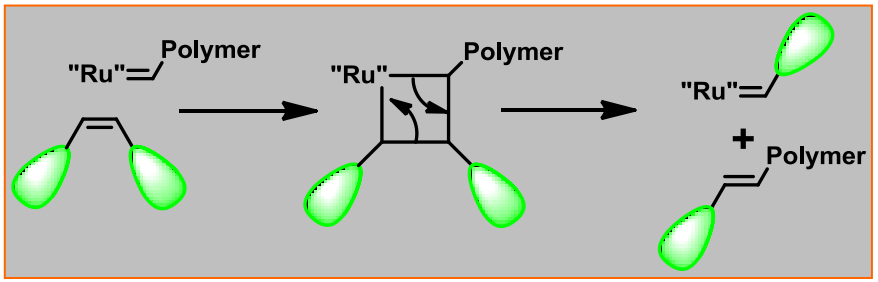


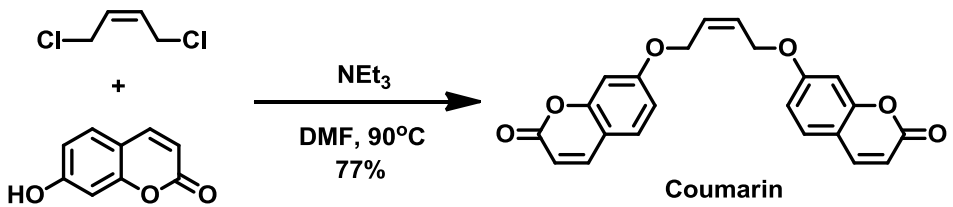



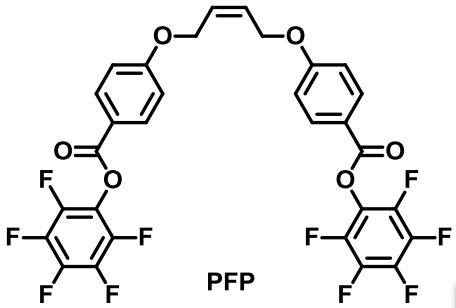

***Miao-Ping Chien,*** *Chem. Sci.* **2012**

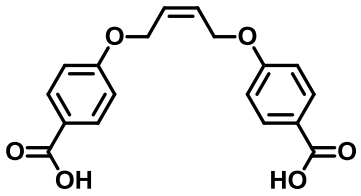
# Terminating Agents for the Introduction of Dyes and Key Functional Groups

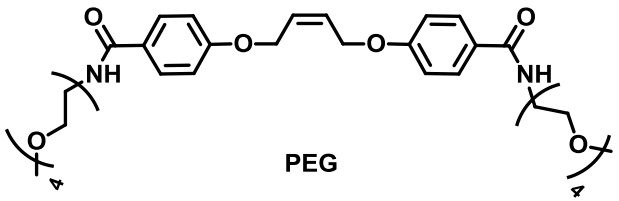
*Matt Thompson, Manuscript in prep*

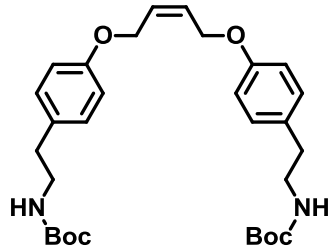


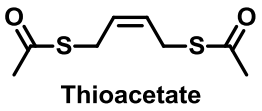



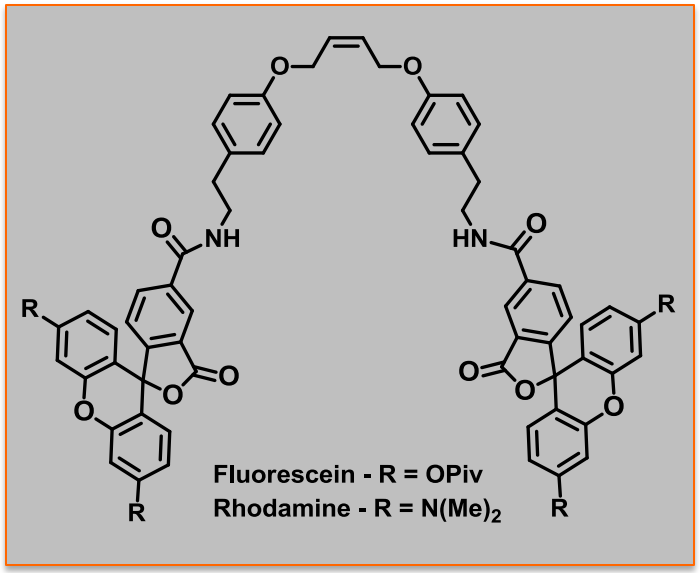



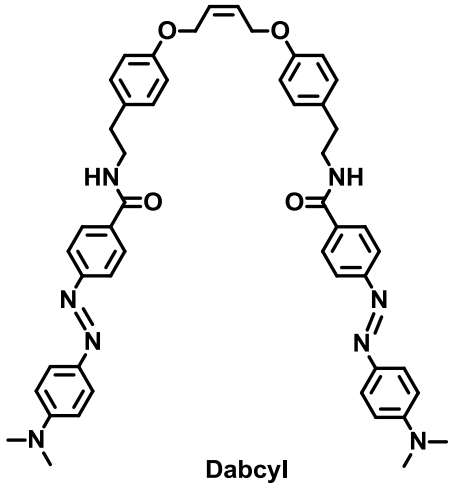



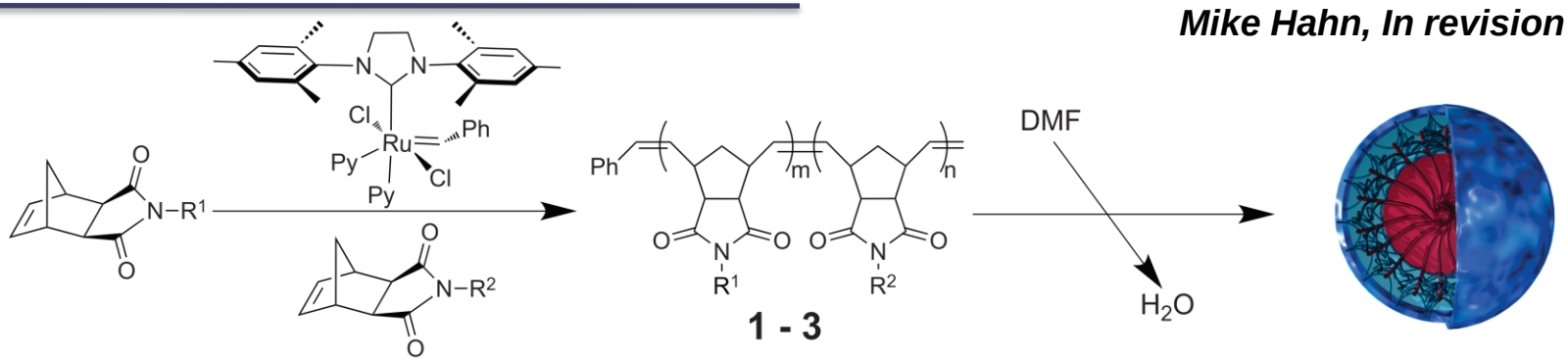



# Peptide-Polymer Amphiphiles (PPAs)

***Mike Hahn, In revision***




**PPAs:**

**1:** $R^1$ = **4**; $R^2$ = **7**

**2:** $R^1$ = **7**; $R^2$ = **5**

**3:** $R^1$ = **6**; $R^2$ = **8**

**Hydrophilic Monomers:**

**4:** -§–Gly-Pro-Leu-Gly-Leu-Ala-Gly-Lys(Ac)-Ebes-$CONH_2$

**5:** -§–Gly-Ebes-Gly-Pro-Leu-Gly-Leu-Ala-Gly-Ebes-$CONH_2$

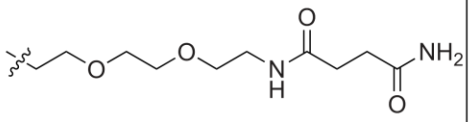
**6:** ≡ OEG

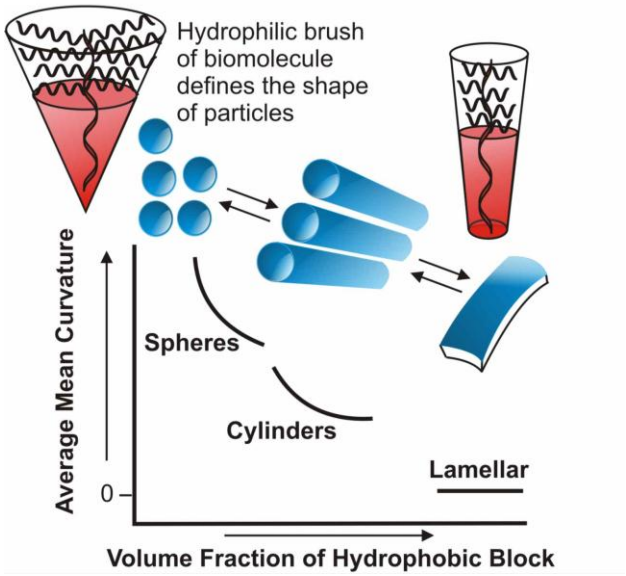
**Hydrophobic Monomers:**

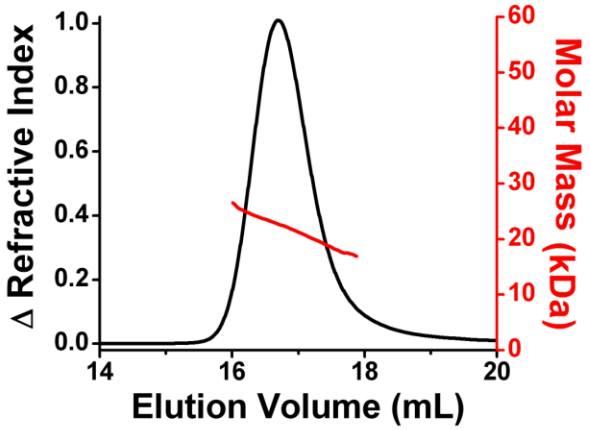
**7:** -§–Ph

**8:** -§–Gly-Phe-Pro-Leu-Ile-$CONH_2$

**Ebes:**



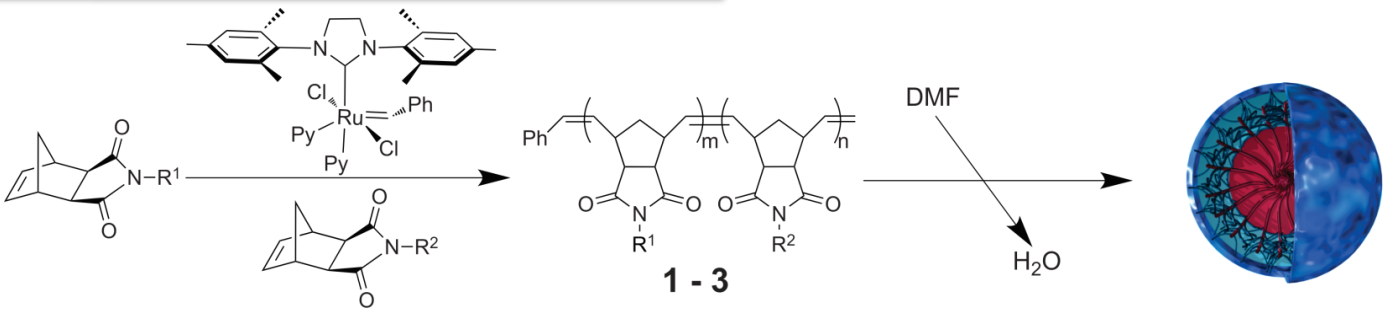

$M_n$ = 22,030 Da

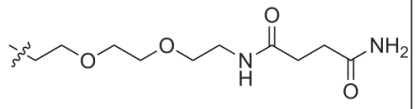
DP = 20

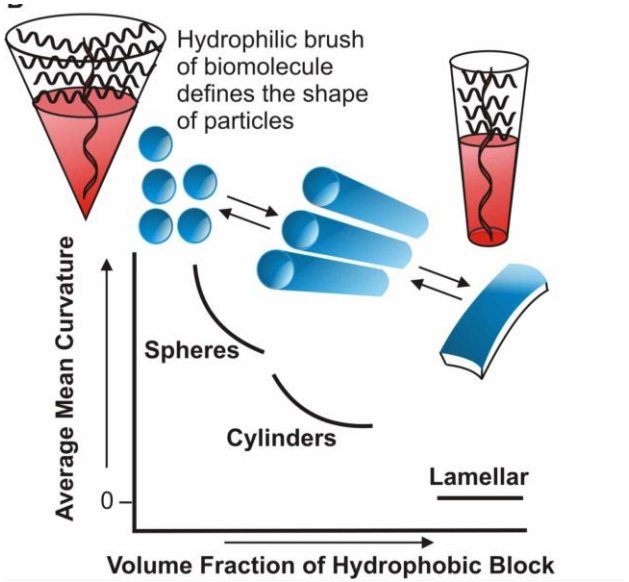
PDI = 1.01

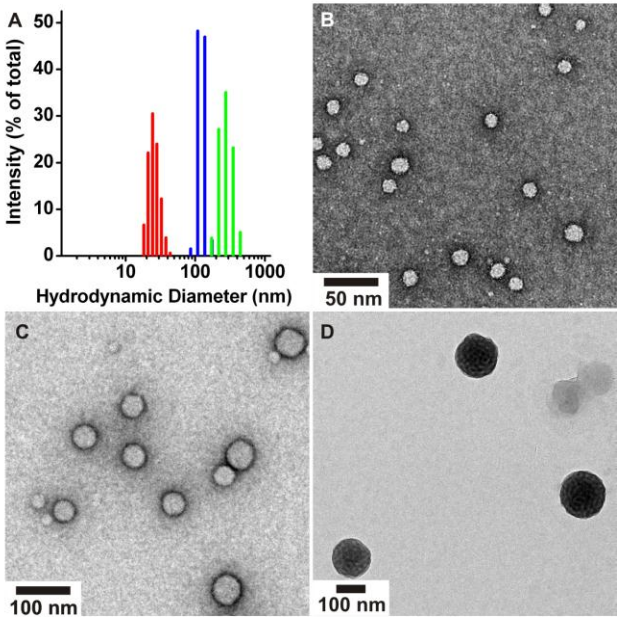

Conrad, R. M.; Grubbs, R. H. *Angew. Chem.* **2009,** *121,* 8478-8480. Biagini, S. C. G. and coworkers *Angew. Chem.* **2008,** *120,* 8991-8994. Roberts, K. S.; Sampson, N. S. *J. Org. Chem.* **2003,** *68,* 2020-2023.

# PPAs Generate Well-defined Micellar Nanoparticles




| PPAs: | Hydrophilic Monomers: | Hydrophobic Monomers: | Ebes: |
|---|---|---|---|
| 1: $R^1$ = 4; $R^2$ = 7 | 4: –Gly-Pro-Leu-Gly-Leu-Ala-Gly-Lys(Ac)-Ebes-$CONH_2$ | 7: –Ph |  |
| 2: $R^1$ = 7; $R^2$ = 5 | 5: –Gly-Ebes-Gly-Pro-Leu-Gly-Leu-Ala-Gly-Ebes-$CONH_2$ | 8: –Gly-Phe-Pro-Leu-Ile-$CONH_2$ | |
| 3: $R^1$ = 6; $R^2$ = 8 | 6: ≡ OEG | | |

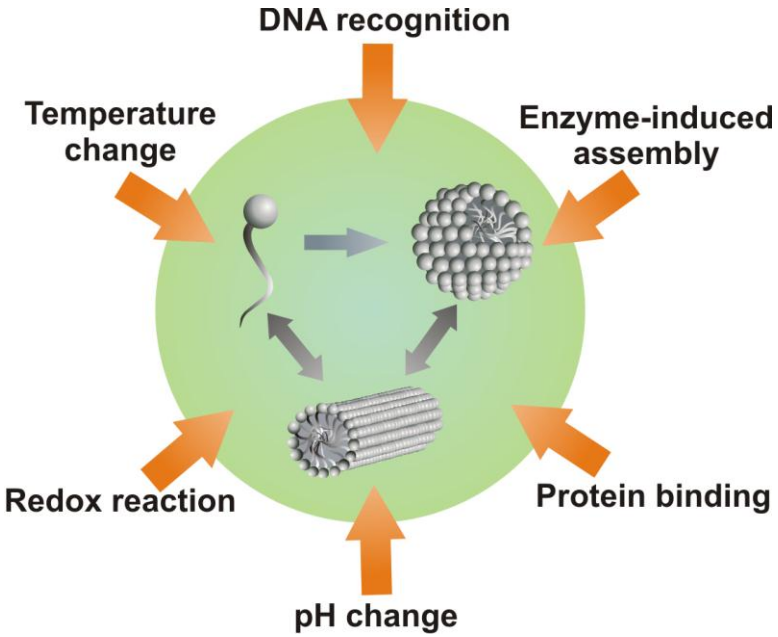


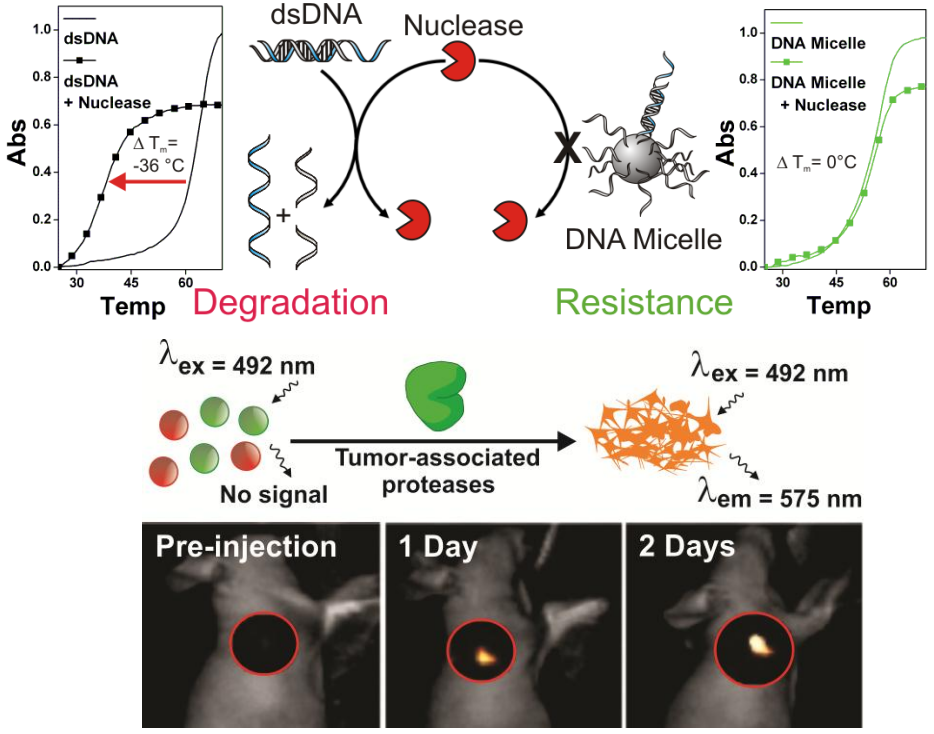

*Mike Hahn, In revision*

# Adaptable, Autonomous Chemical Systems *Basic development and functional application*

**Selective interactions, and well-defined behavior of complex nanostructures in complex milieu**




**Organizing Biomolecules for Response and/or Resistance**




"Enzyme-directed assembly and manipulation of organic nanomaterials"
Hahn, Gianneschi; *Chem. Commun.* **2011**, 47, 11814-11821

"Biological stimuli and biomolecules in the assembly and manipulation of nanoscale particles"
Randolph, Chien, Gianneschi; *Chem. Sci.* **2012**, 3, 1363-1380

# Interactions with other groups and Organizations

**Autonomous Sensors**
Mike Mayer and team (Michigan)

**Dynamic TEM for mechanism elucidation and development of technique as a tool for soft materials**
Nigel Browning (PNNL)

**Autonomous and remote control in biological milieu**
David Hall, Robert Mattrey (UCSD)

**Stabilized Nucleic Acids**
Eric Tatro (UCSD)

**Functional Catalysts and Materials**
BRI – UCSD – Kubiak, Tezcan, Gilson, Burkart

**Biomolecule Programmed Nanoparticles**

**Self-Assembling Healing Scaffolds**
Karen Christman (UCSD)

**Structure Analysis and Morphology**
Tim Baker (UCSD), NIST and Thomas Epps

**Enzyme-Responsive LCs**
Nick Abbott (Wisconsin)

# Relevant Papers Published/ Submitted in the current review period

- Hahn, M. E.; Gianneschi, N. C. “Enzyme-Directed Assembly and Manipulation of Organic Nanomaterials.” *Chem. Comm.,* **2011**, *47*, 11814-11821
- Randolph, L. M.; Chien, M. –P.; Gianneschi, N. C. “Biological Stimuli and Biomolecules in the Assembly and Manipulation of Nanoscale Polymeric Particles” *Chemical Science* **2012**, *3*, 1363-1380
- Chien, M. –P.; Thompson, M. P.; Lin, E. C.; Gianneschi, N. C. “Fluorogenic Enzyme-Responsive Micellar Nanoparticles” *Chemical Science* **2012**, *3*, 2690-2694.
- Rush, A. M.; Thompson, M. P.; Tatro, E.; Gianneschi, N. C. “Nuclease Resistant DNA via High-Density Packaging as Polymeric Micellar Nanoparticles” *SUBMITTED*.
- Chien, M. –P.; Simberg, D.; Thompson, M.P.; Hayashi, T.; Gray, C.; Gianneschi, N. C. “Programming Pharmacokinetics in vivo via Remotely Controlled Switching of Nanoparticle Morphology” *SUBMITTED.*
- Chien, M. –P.; Thompson, M. P.; Barback, C. V.; Hall, D. M.; Gianneschi, N. C. “Enzyme-Directed Assembly of a Nanoparticle Probe in Tumor Tissue” *SUBMITTED*


Patent

- Provisional Patent Application 2012-207: New Methods for Arranging and Packaging Nucleic Acids for Unusual Resistance to Nucleases and Targeted Delivery for Gene Therapy: Gianneschi, Tatro, Rush, **2012**.

# Scientific or Technological Transitions

- Materials developed in this program for enzyme-response have been transitioned into the hands of experts in bioengineering at UCSD (Christman, UCSD)

- Polymer-Peptide Amphiphiles studied in the context of enzyme responsive LC systems (Abbot, Wisconsin)

- Interest in technology related to programmable morphologies for medicinal applications (Novartis)

# Presentations, Activities and Awards, 2012

**Leveraged research funding and awards, 2012**

- NIH – TR01 – Director's Transformative Research Award in collaboration with Prof. Karen Christman, UCSD
- Alfred P. Sloan Foundation Fellow, 2012
- BRI – UCSD Team – Autonomously Evolving Biocatalysts and Functional Materials (PI: Gianneschi)
- BRI in collaboration with U. of Michigan – Disposable, Autonomic Sensors (PI: Mayer)

**Selected Presentations, 2012 (Greater than 25 in 2012 including invited university seminars, and conferences)**

- **Gianneschi, N. C.;** "Enzymes, Peptides and Nucleic acids for Programming the Morphology of Nanoparticles" University of Adelaide, Australia. Invited Seminar. August 2012.
- **Gianneschi, N. C.;** "Enzymes, Peptides and Nucleic acids for Programming the Morphology of Nanoparticles" University of New South Wales, Australia. Invited Seminar. August 2012.
- **Gianneschi, N. C.;** "Enzymes, Peptides and Nucleic acids for Programming the Morphology of Nanoparticles" University of Sydney, Australia. Invited Seminar. August 2012.
- **Gianneschi, N. C.;** "Enzymes, Peptides and Nucleic acids for Programming the Morphology of Nanoparticles" University of Melbourne, Australia. Invited Seminar. August 2012.
- **Gianneschi, N. C.;** "Enzymes, Peptides and Nucleic acids for Programming the Morphology of Nanoparticles" Monash University, Australia. Invited Seminar. August 2012.
- **Gianneschi, N. C.;** "Peptide, DNA, Proteins and Enzymes for Programming Nanomaterials" Warwick Polymer Conference, UK. Invited Seminar. July 2012.
- **Gianneschi, N. C.;** "Programming Polymers with DNA, Peptides and Enzymes" Imperial College, UK. Invited Seminar. May 2012.
- **Gianneschi, N. C.;** "Programming Polymers with DNA, Peptides and Enzymes" ARO Chemical Systems Workshop, Cambridge University, UK. Invited Seminar. May 2012.
- **Gianneschi, N. C.;** "Programming Polymers with DNA, Peptides and Enzymes" Strathclyde University, UK. Invited Seminar. May 2012.
- **Gianneschi, N. C.;** "Programmable Polymeric Nanoparticles" Manchester University, UK. Invited Seminar. May 2012.
- **Gianneschi, N. C.;** "Programmable Nanomaterials" Warwick University, UK. Invited Seminar. May 2012.
- **Gianneschi, N. C.;** "Programming and Switching the Morphology of Polymeric Nanoparticles with DNA, Peptides and Enzymes" Synthetic Chemical Systems Workshop, ARO - Washington, D.C. Invited Talk. April 2012.
- **Gianneschi, N. C.;** "Programmable Nanoscale Materials" Georgia Tech, Atlanta, GA. Invited Seminar. April 2012.
- **Gianneschi, N. C.;** "Programmable Nanomaterials" Emory University. Invited Seminar. April 2012.
- **Gianneschi, N. C.;** "Biomolecular Programming of Discrete Nanomaterials: The Development of Novel Characterization methods for Studying Micellar Nanoparticle Morphology and Stability" American Chemical Society National Meeting, San Diego, CA. Invited Talk. March 2012.

**Other Activities:**

- Stimuli-Responsive Self Assembled Materials Symposium organizer – Polymer Materials Science and Engineering Division Symposium, ACS San Diego, 2012
- Adaptive, Autonomous Soft Materials Symposium co-organizer – MRS, National Meeting, San Francisco, 2013

# Gianneschi Group + Collaborators/Interactions

## Current Students, Postdoctoral Fellows and Research Scientists

| | |
|---|---|
| **Lisa Adamiak** | **(Grad student)** |
| **Sarah Barnhill** | (Grad student) |
| **Miao-Ping Chien** | (Grad student) |
| Carrie James | (Grad student) |
| **Ti-Hsuan Ku** | (Grad student) |
| **Jacquelin Kammeyer** | (Grad student) |
| Clare LeGuyader | (Grad student) |
| **Steven Nguyen** | (Grad student) |
| Swagat Sahu | (Grad student) |
| Lyndsay Randolph | (Grad student) |
| **Anthony Rush** | (Grad student) |
| Kate Veccharelli | (Grad student) |
| Cassi Callmann | (Grad student) |
| Benjamin Monson | (Grad student) |

| | |
|---|---|
| Dr. Angela Blum | (Postdoctoral fellow) |
| Dr. Michael Hahn | (Clinical/Research Resident and Postdoc) |
| Dr. Maria Proetto | (Postdoctoral fellow) |
| **Dr. Matthew Thompson** | (Project Scientist) |
| Lizanne Koch | (Undergraduate researcher) |
| **Alfred Tam** | (Undergraduate researcher) |
| **Alex Caldwell** | (Undergraduate researcher) |
| Dustin Crystal | (Undergraduate researcher) |

## Collaborators – AFOSR Programs

- **Profs Burkart, Gilson, Kubiak, Tezcan**
  BioAutoCatalysis Program
- **Profs Yang** (UCSD), **Mayer, Shtein, and Sept** (U. Mich)
  Autonomic Ion Channel Sensor Program
- **Drs Nigel Browning, James Evans**
  (Pacific Northwest National Labs)
  *In situ* and dynamic TEM (DTEM)
- **Prof Akif Tezcan**
  Dynamic Protein-hybrid materials - DTEM
- **Prof Tim Baker, Norm Olson**
  Cryo-TEM + reconstructions
- **Prof Nick Abbott, Derek Ma** (Wisconsin University)
  Enzyme-responsive LCs

**Hugh De Long, AFOSR**
**PECASE – *FA9550-11-1-0105***