REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

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1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
09-02-2015	Final Report	1-Aug-2011 - 31-Jul-2014

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Final Report: Signal Propagation and Detection via Catalytically Immolative Biopolymer-Programmed Nanomaterials	W911NF-11-1-0264
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER 611102
6. AUTHORS Nathan C. Gianneschi	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES	8. PERFORMING ORGANIZATION REPORT NUMBER
University of California - San Diego 9500 Gilman Drive MC 0934 La Jolla, CA 92093 -0934	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)	10. SPONSOR/MONITOR'S ACRONYM(S) ARO
U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211	11. SPONSOR/MONITOR'S REPORT NUMBER(S) 59784-CH.12

12. DISTRIBUTION AVAILIBILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

Over the course of the funding of this proposal (August, 2011 to March 2013) we endeavored to pioneer the use of selective biochemical reactions and interactions for the preparation and manipulation of nanomaterials. Such interactions, especially those caused by selective enzymatic reactions, are vastly underutilized in materials science in general. This is surprising, given the fact that selective enzymatic reactions are responsible for the formation of some of the most extraordinary examples of well-defined nanomaterials and dynamic processes known from the formation of virus capsids, to the repair and replication of genetic information in living systems. We believe such

15. SUBJECT TERMS

Responsive polymers, nanomaterials, peptides, DNA

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Nathan Gianneschi
UU	UU	UU	UU		19b. TELEPHONE NUMBER 858-246-0857

Report Title

Final Report: Signal Propagation and Detection via Catalytically Immolative Biopolymer-Programmed Nanomaterials

ABSTRACT

Over the course of the funding of this proposal (August, 2011 to March 2013) we endeavored to pioneer the use of selective biochemical reactions and interactions for the preparation and manipulation of nanomaterials. Such interactions, especially those caused by selective enzymatic reactions, are vastly underutilized in materials science in general. This is surprising, given the fact that selective enzymatic reactions are responsible for the formation of some of the most extraordinary examples of well-defined nanomaterials and dynamic processes known from the formation of virus capsids, to the repair and replication of genetic information in living systems. We believe such approaches will enable the preparation of ever more complex nanomaterials capable of adapting to their environment and responding to stimuli in an autonomous fashion yielding switches and changes in their properties, chemistry and function. One particular focus of this program has been an effort to directly incorporate nucleic acids into polymeric materials through polymerization of "nucleic acid monomers", and the incorporation of peptides and sensing elements into responsive nano materials in general. With the completion of this grant we believe we have achieved this, and published on this data accordingly. Excitingly, the work has lead in many new directions and we continue to push forward and make progress in these arenas.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
02/08/2015	8.00	Carrie R. James, Anthony M. Rush, Thomas Insley, Lela Vukovi?, Lisa Adamiak, Petr Král, Nathan C. Gianneschi. Poly(oligonucleotide), Journal of the American Chemical Society, (08 2014): 0. doi: 10.1021/ja503142s
02/08/2015	11.00	Matthew P. Thompson, Lyndsay M. Randolph, Carrie R. James, Ashley N. Davalos, Michael E. Hahn, Nathan C. Gianneschi. Labelling polymers and micellar nanoparticles via initiation, propagation and termination with ROMP, Polymer Chemistry, (2014): 0. doi: 10.1039/c3py01338c
02/08/2015	10.00	Anthony M. Rush, Miao-Ping Chien, Patricia Abellan Baeza, Joseph P. Patterson, Matthew P. Thompson, Norman H. Olson, Curtis E. Moore, Arnold L. Rheingold, Maria T. Proetto, Christopher Andolina, Jill Millstone, Stephen B. Howell, Nigel D. Browning, James E. Evans, Nathan C. Gianneschi. Dynamics of Soft Nanomaterials Captured by Transmission Electron Microscopy in Liquid Water, Journal of the American Chemical Society, (01 2014): 0. doi: 10.1021/ja408513m
02/08/2015	9.00	Angela P. Blum, Jacquelin K. Kammeyer, Jian Yin, Dustin T. Crystal, Anthony M. Rush, Michael K. Gilson, Nathan C. Gianneschi. Peptides Displayed as High Density Brush Polymers Resist Proteolysis and Retain Bioactivity, Journal of the American Chemical Society, (10 2014): 0. doi: 10.1021/ja5088216
09/12/2012	2.00	Lyndsay M. Randolph, Miao-Ping Chien, Nathan C. Gianneschi. Biological stimuli and biomolecules in the assembly and manipulation of nanoscale polymeric particles, Chemical Science, (01 2012): 1363. doi: 10.1039/c2sc00857b
09/12/2012	1.00	Matthew P. Thompson, Eugene C. Lin, Miao-Ping Chien, Nathan C. Gianneschi. Fluorogenic enzyme-responsive micellar nanoparticles, Chemical Science, (06 2012): 2690. doi: 10.1039/c2sc20165h
09/12/2012	3.00	Michael E. Hahn, Nathan C. Gianneschi. Enzyme-directed assembly and manipulation of organic nanomaterials, Chemical Communications, (09 2011): 11814. doi: 10.1039/c1cc15220c
10/01/2013	4.00	Matthew P. Thompson, Erick T. Tatro, Nathan C. Gianneschi, Anthony M. Rush. Nuclease-Resistant DNA, ACS Nano, (02 2013): 3599. doi: 10.1021/nn305030g
10/01/2013	5.00	Michael E. Hahn, Lyndsay M. Randolph, Lisa Adamiak, Matthew P. Thompson, Nathan C. Gianneschi. Polymerization of a peptide-based enzyme substrate, Chemical Communications, (02 2013): 2873. doi: 10.1039/c3cc40472b
10/01/2013	6.00	Jacquelin K. Kammeyer, Angela P. Blum, Lisa Adamiak, Michael E. Hahn, Nathan C. Gianneschi. Polymerization of protecting-group-free peptides via ROMP, Polymer Chemistry, (03 2013): 3929. doi: 10.1039/c3py00526g
10/01/2013	7.00	Miao-Ping Chien, Matthew P. Thompson, Christopher V. Barback, Ti-Hsuan Ku, David J. Hall, Nathan C. Gianneschi. Enzyme-Directed Assembly of a Nanoparticle Probe in Tumor Tissue, Advanced Materials, (07 2013): 3599. doi: 10.1002/adma.201300823

TOTAL: **11**

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

2011 UCSD Panhellenic Outstanding Professor Award
2011 NIH Director's New Innovator Award
2012 Alfred P. Sloan Foundation Fellow
2012 NIH Director's Transformative Research Award
2013 National Academy of Sciences Kavli Fellow

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Carrie James	1.00	
FTE Equivalent:	**1.00**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Yiwen Yi	1.00
FTE Equivalent:	**1.00**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Nathan Gianneschi	0.25	
FTE Equivalent:	**0.25**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Technology Transfer

Annual Progress Summary

Subject: Annual Progress Statement to Dr. Jennifer J. Becker

Contract/Grant Title: Signal Propagation and Detection via Catalytically Immolative Biopolymer-Programmed Nanomaterials

Contract/Grant #: W911NF-11-1-0264

Reporting Period: 01/08/2011 – 31/7/2014

Brief Summary of Annual Accomplishments:

Over the course of this award our research program has progressed on three key fronts. As an overview, in an effort to apply what we have learned regarding enzymatically responsive materials, we have demonstrated our systems capabilities as tumor targeting materials in a range of animal models for human cancer (Advanced Materials, 2013). This kind of directed assembly within tissue is unprecedented and has been made possible through our approach to utilizing highly selective interactions coupled with new approaches to biopolymer preparation. Therefore, the three key advancements have been: 1) Application of materials to selective sense-and-response systems in highly complex environments (Advanced Materials, 2013). 2) New polymerization approaches (Polymer Chemistry, 2013 and Chemical Communications, 2013). 3) Stabilization of biomolecules within nanoparticle frameworks, with an emphasis on nucleic acid stabilized structures (ACS Nano, 2013). 4) The direct polymerization of PNA-based norbornyl monomers as an initial example of a new type of informational brush polymer. These collective efforts are expected to impact how synthetic materials are coupled with biomolecules to achieve adaptive and unique function in sense-and-response systems.

Through the publication of primary research articles, and presentations at top institutions worldwide and at conferences, we have aimed to establish biomolecules as tools for the programmed manipulation of nanomaterials. This is an important and powerful contribution to the field of nanoscale synthesis in general, and in the development of truly informational materials capable of interfacing with biological systems specifically. These adaptable, autonomic chemical systems are are now being utilized for detection strategies and in the assembly well-defined nucleic acid hybrid polymer materials. Furthermore, these projects have lead to collaborations with both Prof. Nick Abbott at Wisconsin and Prof. Thai Thayumanavan at UMass, Amherst aimed to developing new methods for propagating chemical responses through multiple length scales.

Archival Publications (published) during the funding period:

1) Hahn, M. E.; Gianneschi, N. C. “Enzyme-directed assembly and manipulation of organic nanomaterials” *Chemical Communications*, **2011**, *47*, 11814-11821

2) Chien, M. –P.; Thompson, M. P.; Lin, E. C.; **Gianneschi, N. C.** “Fluorogenic Enzyme-Responsive Micellar Nanoparticles” *Chemical Science* **2012**, *3*, 2690-2694.
3) 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.
4) Chien, M. –P.; Thompson, M. P.; Barback, C. V.; Hall, D. J.; **Gianneschi, N. C.** “Enzyme-Directed Assembly of a Nanoparticle Probe in Tumor Tissue” *Advanced Materials* **2013**, *25*, 3599-3604
5) Kammeyer, J. K.; Blum, A. P.; Adamiak, L. Hahn, M. E.; **Gianneschi, N. C.** “Polymerization of Protecting-Group-Free Peptides via ROMP” *Polymer Chemistry* **2013**, *4*, 3929-3933
6) Hahn, M. E.; Randolph, L. M.; Adamiak, L.; Thompson, M. P.; **Gianneschi, N. C.** “Polymerization of a Peptide-Based Enzyme Substrate” *Chem. Commun.* **2013**, *49*, 2873-2875
7) Rush, A. M.; Thompson, M. P.; Tatro, E. T.; Gianneschi, N. C. “Nuclease-resistant DNA via High Density Packing in Polymeric Micellar Nanoparticle Coronas” *ACS Nano* **2013**, *7*, 1379-1387
8) Thompson, M. P.; Randolph, L. M.; James, C. R.; Davalos, A. N.; Hahn, M. E.; Gianneschi, N. C. “Labelling polymers and micellar nanoparticles via initiation, propagation and termination with ROMP” *Polymer Chemistry* **2013**, *5,* 1954-1964
9) James, C. R.; Rush, A. M.; Insley, T.; Vukovic, L.; Kral, P.; **Gianneschi, N. C.** “Poly(oligonucleotide)” *J. Am. Chem. Soc.* **2014**, *136*, 11216-11219.
10) Proetto, M. T.; Rush, A. M.; Chien, M. –P.; Abellan Baeza, P.; Patterson, J. P.; Thompson, M. P.; Olson, N. H.; Moore, C. E.; Rehingold, A. L.; Andolina, C.; Millston, J.; Howell, S. B.; Browning, N D.; Evans, J. E..; **Gianneschi, N. C.** “Dynamics of Soft Materials Captured by Transmission Electron Microscopy” *J. Am. Chem. Soc..* **2014**, *136,* 1162-1165.

Changes in research objectives: None

Change in ARO program manager: None

Extensions granted or milestones slipped: None

Patent Disclosures in this reporting period:
1) **Gianneschi, N. C**.; Rush, A. M. Tatro, E.; Packaged Nucleic Acids. SD2012-207

Detailed Description of Accomplishments over the past 12 months

Stated Objectives for 3 year period

1) *Objective 1:* To develop and understand surface-enhanced catalysis within DNA-based shells of biohybrid polymeric micelles.

2) *Objective 2:* To develop chemically reactive micelle cores for morphology directed release of payload.

3) Objective 3: To develop the concept of enzyme directed assembly and disassembly of micellar assemblies as propagators of stimuli-induced enzymatic cascade reactions.

Findings over reporting period with respect to the core kinds of materials

DNA-based shells of biohybrid polymeric micelles

In summary, towards the development of DNA-based materials, where interactions at their surface propagate a catalytic response, we made progress on several key fronts. We began with an observation of unusual catalytic activity of a DNAzyme on a DNA-based micelle surface (Chem Comm, 2011). We followed this work with that described in our 2013 ACS Nano paper. This work involved a novel approach for rendering DNA resistant to two key classes of nuclease that are otherwise capable of rapidly degrading substrates in a sequence-selective or non-selective fashion. Inspiration for this investigation was drawn from the increasing interest in novel approaches for packaging and delivering nucleic acids for *in vivo* applications and for incorporation into devices reliant on long-lasting nucleic acids.[1-5] This interest has led to an array of materials designed to facilitate potent and selective communication with important cellular machinery and recognition elements.[6-13] Our approach is predicated on the idea that a key requirement for any enabling technology of this type is a well-defined nucleic-acid based material that maintains the integrity of the base-sequence in nuclease-rich environments commonly the case in real-world systems. Indeed, these observations regarding resistance came about somewhat contrary to our initial goals, which involved observing enzymatic reactions at the surface. Ongoing work in our laboratory seeks to decipher the mechanism of resistance, and to understand how it can be tuned.

One of the main achievements that has built upon our work on nucleic acids over the past several years has been the development of a method for directly polymerizing them into a polymer and on to a polymeric micelle. This has, in part been motivated by a desire to tightly control the incorporation of informational biomolecules into the surface of structured nanoparticles. Of course, the display of chemical functionality in a multivalent fashion on surfaces, particles, and as brushes on polymer backbones is a common theme in nature as well as for synthetic systems.[14-16] Such systems take advantage of the unique properties that arise when monomeric species are incorporated

into a densely packed three-dimensional architecture. In this work, the preparation of polymeric nucleic acids wherein single-stranded sequences of peptide nucleic acids (PNAs) are incorporated as polymer brushes *via* graft-through polymerization using the ROMP initiator $(IMesH_2)C_5H_5N)_2$-$(Cl)_2$Ru=CHPh. Nucleic acids, both natural and synthetic, standout as the quintessential carriers of chemical information stored as specific sequences of bases positioned along a backbone.[16-19] As such, synthetic oligonucleotides and nucleic acid bioconjugates are powerful tools in a range of fields including in biotechnology (e.g. PCR)[20,21], in materials science as programmable structural synthons[22-28] and as aptamers selected by *in vitro* evolution.[29-33] In each application the nucleic acid functions to enrich a chemical system with information, facilitating predictable interactions with complementary sequences[34] or with other molecules including enzymes, proteins, and small molecules.[30,35-37] We reasoned an approach allowing the graft-through polymerization of an oligonucleotide sequence would provide a powerful new tool for the multivalent display of chemical information on a synthetic template. Therefore, we have shown that one can prepare nucleic acid brush polymers and amphiphilic brush copolymers by direct polymerization via graft-through polymerization of a nucleic acid. This is an example of polymer-nucleic acid bioconjugates generated via direct polymerization of nucleic acid-based monomers. In addition, these materials show cooperative hybridization to complimentary DNA oligonucleotides. We believe this type of approach provides an efficient synthetic strategy for the incorporation of nucleic acids into particle and polymer-based materials. The interest in doing so is driven by potential applications including the facile preparation of materials for affinity purification of DNA,[38,39] gene and nucleic acid delivery to cells[17,40-43,2,44,45] and in the development of materials capable of programmed self-assembly[24-28,46-49,50].

Chemically reactive micelle cores

Towards this class of materials, we have developed Pt(II)-based nanoparticle system that undergoes timed release of a core-bound material. Related systems undergo release of payloads in response to enzymatic action at the particle shells combined with small molecule stimuli. One of the exceptional outcomes to arise from these efforts involves our work on in situ TEM (*J. Am. Chem. Soc..* **2014**, *136,* 1162-1165). The high contrast, platinum cores provided enough contrast for us to observe motion of soft nanoparticles for the first time in this work. Therefore, in this paper, we demonstrate for the first time, the use of *in situ* TEM to characterize soft materials. This technique has been available for some time, but only in recent years has the technology been useful for such studies. However, it has never been employed to look at soft, synthetic materials despite the fact that this is exactly the class of materials that will benefit greatly from such a tool where dynamics are absolutely critical. We believe this is likely due to the fact that the technology is relatively new in its current form, and that it is only now emerging as a viable technique. This makes any demonstration of it as a tool for imaging dynamics on the nanometer length scale, a potentially very high impact contribution to materials chemistry. There could be no more timely a demonstration than now, as the tool becomes available, and researchers become ever more aware that

they need to understand dynamics, and far-from-equilibrium systems in order to grasp the true nature of the materials they work with.

A general problem in nanomaterials research is characterization. Especially useful parts of the solution are the multiple TEM techniques where sufficient resolution is obtained, but artifacts are a fact of life. However, to date, these techniques have been limited to dry-state, high vacuum, or cryogenically preserved samples in ice. Indeed, combinations of these are used in order to separate truth from fiction as often as possible! Obviously, none of these standard techniques is useful for imaging particles in their natural state: i.e. freely moving in solution. We find this to be perhaps the largest gap in capability facing this broad field of research. Indeed, inorganic materials have begun to be imaged in situ (because of the high contrast from high Z-elements), but even for these systems, such studies are still rare. Again, this is not a state of affairs that is bound to last very long, as researchers become more interested in imaging/characterizing native state materials in a dynamic fashion. We should add here, that the enormous number of hydrogels that are being investigated have never been imaged in solution, only in high vacuum; *in situ* TEM will be perfect for these studies where the majority of the material is indeed, liquid water, meaning that any high vacuum EM image is likely nothing like the real structure and can only be used as a rough guide at best.

In this work we achieved this imaging capability for the first time, by employing a particle we have been developing for *in vivo* therapy against ovarian cancer. The particles consist of a Pt(II)-based core, that is the heart of the anticancer properties of the materials. They are currently being tested *in vitro*, and will be moved into animal models later together with co-author Stephen Howell, an oncologist at UCSD and an expert in intraperitoneal treatment of ovarian cancer. We had been trying to find conditions to image in water by TEM, and tried these particles because of their fantastic contrast in TEM. They serendipitously provided the contrast enabling easy imaging. We do not focus on their novelty in the paper as published (*JACS*), but they are the first example of a polymerized Pt(II) drug analogue, and we believe this type of material will have great efficacy. However, in this work we use it for its contrast. Despite that fact, we believe we will be able to image non-heavy-metal containing particles in the future, as we hone the technique in solution. Therefore, this is not a limitation on generality, just an initial, easily approachable system for proof-of-concept purposes.

We should note that our own research within this ARO-funded program has been focused on developing stimuli responsive systems that undergo dramatic changes in three-dimensional morphology. As with any other lab, we have relied on taking “snapshots” in the dry state, often stained with heavy metals, in order to elucidate these morphology changes via TEM. Obviously this is a far cry from actually imaging dynamics. Therefore, we purchased an *in situ* cell holder for our TEM, in order to conduct this type of study as part of routine analysis. These cell systems will allow multi-channel loading of samples in flow mode into the TEM, such that conditions can be varied *during* the imaging process. Simply put, this technique will revolutionize how we study dynamic, switchable materials and will greatly alter expectations for how such

materials should be handled and imaged. Indeed, we anticipate *in situ* analysis will rapidly become one of the multiple, gold standard TEM techniques but the only one capable of capturing materials far from equilibrium and in a solvated state. This is an exciting time for TEM and this paper sits at the frontier. Indeed, in an ongoing set of studies we are attempting to observe metal-organic framework formation, with initial success.

Enzyme directed assembly and disassembly of micellar assemblies as propagators of stimuli-induced enzymatic cascade reactions.

In our work, we have shown that nucleic acids have exceptional properties with regards to enabling the predictable formation polymeric nanostructures with unusual recognition and reactivity patterns while maintaining informational integrity. However, nucleic acids can be difficult to scale up from a chemistry perspective and from the shear expense of doing so (hence our move towards PNA nanostructures as described briefly above). Certainly, if the applications warrant this, it can be done. However, we are interested in the possibility of achieving a similar level of programmability with peptides and proteins that can be generated in higher yield, and are theoretically more stable.

This part of our program has as its overarching goal the development of methods for programming nanomaterials to perform complex changes in structure and function in response to environmental cues. This has lead to the design and implementation of novel DNA- and peptide-programmed micellar nanoparticles that can be deployed for sense-and-response applications. This program has formed the backbone of our group's efforts to establish techniques for building complexity into nanomaterials through robust methodologies in materials science and polymer chemistry. It also has as one of its key goals, the development of novel characterization methods for analysis of nanomaterials in complex milieu from biological fluids, to organs to environmental samples. Moreover, we are increasingly interested in the characterization of nanoparticles undergoing controlled switches and changes in structure and function in a *dynamic* fashion. Elucidation of parameters governing dynamics will be the next frontier for this class of functional material. This work involves a collaboration with Dr. James Evans and Dr. Nigel Browning to examine dynamic, switchable materials in solution phase via novel in situ, and dynamic TEM techniques (work being conducted at UCSD and at EMSL – PNNL). In addition, the project involves a small-angle neutron scattering (SANS) study that will soon be underway at the Lujan Neutron Scattering Center at Los Alamos National Lab. This effort is aimed at comparing cryo-TEM reconstructed data and simulated structures to information obtained via SANS. In addition, this project involves the development of chemical systems capable of selectively sensing small molecule and biomolecular stimuli, and then responding through changes in materials properties over multiple length scales. This is a significant challenge in chemistry, and relies not just on selective recognition, but on *propagation* of a response. Chemical feedback loops, both positive and negative, are a major area of interest here, as are templating and replicative mechanisms of action in pursuit of our goals. This program has several sub-aims including 1) Stabilization of biomolecules in non-natural and harsh environments such that they can be deployed in the real world as components in sense-

and-response systems. 2) Reactive, informational systems for signal propagation over multiple length scales. This effort has spawned multiple collaborations including one with Prof. Nick Abbott at Wisconsin in the development of novel sense-and-response liquid crystals.

In the following sections, we will highlight the key findings of our papers on this general area that were published in this reporting period:

Summary

We have described our work over the past several years of funding from this grant in the study of nucleic acid and peptide-based nanoparticles that undergo enzyme-induced changes in structure, or remain intact with remarkable resistance to selective and persistent attack by natural enzymes. The capabilities of these materials is underlined clearly by the ability of probe technologies we have developed to work in complex milieu, including within living organisms. This is extremely encouraging for this platform as a sense-response system capable of autonomous sensing in the environment. In addition, we have demonstrated that soft matter can be observed undergoing dynamic motion utilizing in situ TEM, a technique that we aim to continue to develop for the observation of responsive materials in general.

Supported Personnel

Nathan Gianneschi (PI)
Carrie James (graduate student)
Yiwen Li (postdoctoral fellow)

Interactions/Transitions

Prof. Nigel Browning and James Evans (Pacific Northwest National Labs, EMSL)
- Prof. Browning and James Evans are experts in dynamic TEM and liquid-phase in situ TEM. We aim to study our materials and help in the optimization of this EM approach for the study of the mechanism of transformations of enzyme, DNA, and temperature responsive particles and templation studies.

Prof. David Hall, Dr. Robert Mattrey, and Dr. Stephen Howell (UCSD, Medical School)
–Fluorescence imaging and *in vivo* studies of stimuli responsive materials in drug delivery and in molecular diagnostics

Eric Tatro (UCSD)
–Nucleic acids stabilized in nanostructures for retaining biological function, including gene delivery applications of interest to Novartis.

Tim Baker and team (UCSD, Chemistry & Biochemistry, and Biology)
–Development of Cryo-TEM and image reconstruction for high resolution structures of soft, micellar nanoparticles

Akif Tezcan and team (UCSD, Chemistry & Biochemistry)
-Metal-Directed Semi-synthetic Protein Nanoparticles

Nick Abbott and team (Wisconsin)
-Interfacing nucleic acid and peptide programmed polymers with liquid crystals as an way of propagating stimuli through materials to generate truly responsive systems on the macroscale.

Thai Thayumanavan and team (UMass Amherst)
-We have worked with this team on including high contrast dyes as guests within our materials as part of our Objective 2 of this proposal.

Leveraged research funding and awards

Bio-hybrid Polymers and Micellar Nanoparticles for Stimuli Responsive Materials

Source of Support:	W911NF-13-1-0321 Department of Defense/Army Office of Research (ARO) – Defense University Research Initiative Program (DURIP)
Project Location:	University of California, San DIego
Total Award Amount:	$103,467
Starting Date:	8/01/13
Ending Date:	7/31/14
PI:	**N.C. Gianneschi**

Goal of the project: Equipment grant to support ongoing efforts in the development of adaptive, autonomous chemical systems. Purchasing is ongoing, but will include several instruments and components for high resolution characterization of nanoparticles undergoing dynamic changes in structure and function, in a range of milieu including biological fluids.

Project/Proposal Project/Proposal Title: Adaptive Materials Symposium (Spring MRS, 2013)

Source of Support:	W911NF-13-1-0060 Department of Defense/Army Office of Research (ARO) – Conference grant to support Materials Research Society National Meeting: Adaptive Materials Symposium
Project Location:	University of California, San DIego
Total Award Amount:	$10,000
Starting Date:	4/01/13
Ending Date:	6/30/13
PI:	**N.C. Gianneschi**

Goal of the project: Grant was provided for support of a symposium held at the 2013, Spring MRS meeting in San Francisco concerning the development of advanced adaptive materials. Symposium was organized together with Prof. Rein Ulijn, Strathclyde University, and was co-sponsored by the Royal Society of Chemistry.

Project/Proposal Project/Proposal Title: CCI Phase II, Center for Aerosol Impacts on Climate and the Environment
Source of Support: National Science Foundation
Project Location: University of Utah (project centered at UCSD)
Total Award Amount: $20,000,000
Starting Date: 10/01/13
Ending Date: 09/30/18
PI: K.A. Prather (PI), **N.C. Gianneschi (Senior Personnel)**
Goal of the project: Focus on developing a better understanding of the role of aerosols in impacting climate through research, innovation, education, and informal science communication.
Person-months Per Year Committed to the Project: Cal: 0.0 Acad: 0.45 Summer: 0.0

Project/Proposal Project/Proposal Title: Dynamic Bioresponsive Nanomaterials Captured by Dynamic Transmission Electron Microscopy and High Resolution Optical Microscopy
Source of Support: DOE - Environmental Molecular Sciences Laboratory at the Pacific Northwest National Laboratory
Project Location: EMSL
Total Award Amount: No direct or indirect – Involves extensive instrument access
Starting Date: 2011
Ending Date: 2014 (present year extension was granted on September 5th)
PI: **N.C. Gianneschi (PI),** A. Tezcan (PI)
Goal of the project: The development of dynamic and in situ TEM techniques for characterizing nanomaterials in liquid phase. This work is being done in collaboration with Dr. James Evans and Dr. Nigel Browning at PNNL-EMSL. In addition, we are working with Dr. Galya Orr and Dr. Dehong Hu, in the development of STORM for the super high resolution reconstruction of fluorescence images of particles present in ex vivo samples of tissues from animals treated with enzyme-responsive nanoparticles. As of October 1st, 2013 the following instruments are available for use by our laboratory with some training and professional assistance: STORM/PALM Super-resolution – 160 hrs. Fluorescence Single-Molecule – 100 hrs. Confocal Fluorescence Microscopy, Multi-Photo/FLIM integrated, 120 hrs. Transmission Electron Microscope (for in situ TEM) – 250 hrs. Mass Spectrometry – ICP-MS, Ultra-high resolution – 100 hrs.

Project/Proposal Project/Proposal Title: Probing the Internal Structural Morphology of Complex Biohybrid Polymeric Nanoparticles
Source of Support: DOE – Los Alamos National Laboratory
Project Location: Lujan Neutron Scattering Center
Total Award Amount: No direct or indirect – Involves 10 days of access to SANS
Starting Date: September 2013
Ending Date: 10 days of access granted – 5 to be used, September 2013

PI: **N.C. Gianneschi (PI)**

Goal of the project: The aim is to study our dynamic nanoparticle systems via Small Angle Neutron Scattering. This technique will give valuable structural information to be contrasted and compared to TEM and to atomistic models being developed in collaboration with theorist Petr Kral (UI Chicago).

Selected presentations during funding period

A – Academic and Corporate Institutions

1) **University of Miami, Miami, FL**
   Invited Seminar Sept 2013
2) **University of Florida, Gainesville, FL**
   Invited Seminar Sept 2013
3) **Northwestern University, Evanston, IL**
   Invited Seminar Sept 2013
4) **University of Illinois, Chicago. Chicago, IL**
   Invited Seminar Sept 2013
5) **The Ohio State University, Columbus, OH**
   Invited Seminar Aug 2013
6) **University of Erlangen-Nuremburg, Erlangen, Germany**
   Invited Seminar Aug 2013
7) **University of Zurich, Zurich, Switzerland.**
   Invited Seminar May 2013
8) **Case Western Reserve, Cleveland, OH**
   Invited Seminar April 2013
9) **University of Massachusetts, Amherst, MA**
   Invited Seminar Feb 2013
10) **University of California, Berkeley, CA.**
    Invited Seminar Oct 2012
11) **Purdue University, West Lafayette, IN.**
    Invited Seminar Oct 2012

B – Conference Symposia and Workshops

1) **Transatlantic Frontiers in Chemistry, Kloster Seeon**
   Invited Talk Aug 2013
2) **3rd Chemical Science Symposium on Functional Supramolecular Materials, Hangzhou, China.**
   Invited Talk June 2013
3) **ISACS: Challenges in Organic Materials & Supramolecular Chemistry, Kyoto, Japan.**
   Invited Talk June 2013
4) **Gordon Research Conference: Self-Assembly & Supramolecular Chemistry, Les Diablerets, Switzerland.**
   Invited Talk May 2013
5) **100 Years of Colloidal Science Symposium:**

American Chemical Society National Meeting, New Orleans, LA.
Invited Talk April 2013

6) **Chemical Systems Workshop, ARO, Providence, RI.**
Invited Talk Oct 2012

Other Activities

Symposium/Conference Organizer:

1) **Co-Chair** with Profs. Aoki (Tokyo), Chiu (Tsing Hua, Taiwan) and Soga (Tokyo): Multiscale & Synergistic Supramolecular Systems in Biomedical and Materials Sciences – Pacifichem, Hawaii, 2015
2) **Conference Scientific Chair:** RSC International Symposia on Advancing the Chemical Sciences - ISACS-15, San Diego, CA, August 2014. Topic: Nanoscience.
3) **Co-Chair** with Prof. Ezat Khosravi (Durham University): FUSION Conference on Functional Polymeric Materials – Cancun, Mexico, February 2014
4) **Symposium Organizer:** Adaptive Materials Symposium – Materials Research Society Meeting, San Francisco, 2013

Discussion Leader:

1) **Discussion leader:** RSC International Symposia on Advancing the Chemical Sciences (ISACS-10): Challenges in Organic Materials and Supramolecular Chemistry, Kyoto, Japan, 2013
2) **Discussion leader:** Gordon Research Conference: Supramolecular Chemistry, Barga, Italy, 2011

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