

**FINAL REPORT FOR AWARD # 0203707**

Louis A Lyon ; *GA Tech Res Corp - GIT*
Stimuli-Sensitive Core/Shell Microgels

Participant Individuals:

Graduate student(s) : Clinton D Jones

Post-doc(s) : Daoji Gan

Graduate student(s) : Satish Nayak; Jonathan McGrath

Participants' Detail**Partner Organizations:**

Purdue University: Collaborative Research

Prof. Jean Chmielewski, Chemistry

Other collaborators:

We are collaborating with Jean Chmielewski of Purdue University on the targeted delivery of core/shell particles to cancer cell models. We are preparing folate-labeled particles at GT and they perform the cellular uptake studies.

Activities and findings:**Training and Development:**

One former student (Dr. Clint Jones, Assistant Professor at Mercyhurst College) and a postdoc (Dr. Daoji Gan, E&A, Inc.) contributed to the early work on this project. They were followed by, four graduate students: Dr. Satish Nayak (Postdoc at University of Chicago), Bart Blackburn (current 4th year student), Jonathan McGrath (current 5th year student), and Neetu

Singh (current 4th year student). Undergraduates Christina Baker (female Latino student) and Robert Bibb have also been involved in these studies. Christina Baker was awarded a GT Summer Research Fellowship for her work on this project; she is currently in the chemistry Ph.D. program at UCLA. Kristin Shepherd (African-American woman, Lincoln University) participated in this work through the GT NSF-REU program. The graduate students working on this project have received an education in polymer and particle synthesis, electron and light microscopy, fluorescence techniques, organic synthesis (new monomers), light scattering methods, and NMR.

Outreach Activities:

We are attempted to involve local high school teachers in these projects through the RET program. Unfortunately, this program was ended at GT, and supplemental funds were not available through the DMR program.

Journal Publications:

- Jones, C. D.; Lyon, L. A., "Shell-Restricted Swelling and Core Compression in Poly(N-Isopropylacrylamide) Core-Shell Microgels", *Macromolecules*, vol. 36, (2003), p. 1988. Published
- Gan, D.; Lyon, L. A., "Synthesis and Protein Adsorption Resistance of PEG-Modified Poly-N-isopropylacrylamide Core/Shell Microgels", *Macromolecules*, vol. 35, (2002), p. 9634. Published
- Daoji Gan; L. Andrew Lyon, "Fluorescence Nonradiative Energy Transfer Analysis of Crosslinker Heterogeneity in Core-Shell Hydrogel Nanoparticles", *Analytica Chimica Acta*, vol. 496, (2003), p. 53. Published
- Jones, C. D.; Lyon, L. A., "Dependence of Shell Thickness on Core Compression in Acrylic Acid Modified Poly(N-isopropylacrylamide) Core/Shell Microgels", *Langmuir*, vol. 19, (2003), p. 4544. Published
- Nayak, S.; Lyon, L. A., "Photoinduced Phase Transitions in Poly(N-isopropylacrylamide) Microgels", *Chemistry of Materials*, vol. 16, (2004), p. 2623. Published
- Jones, Clinton D.; McGrath, Jonathan G.; Lyon, L. Andrew, "Characterization of Cyanine Dye-Labeled Poly(N-isopropylacrylamide) Core/Shell Microgels Using Fluorescence Resonance Energy Transfer", *Journal of Physical Chemistry B*, vol. 108, (2004), p. 12652. Published
- Nayak, S.; Lyon, L. A., "Ligand-Functionalized Core/Shell Microgels with Permselective Shells", *Angew. Chem. Int. Ed.*, vol. 42, (2004), p. 6706. Published
- Satish Nayak; Daoji Gan; Michael J. Serpe; L. Andrew Lyon, "Hollow Thermoresponsive Microgels", *Small*, vol. 1, (2005), p. 416. Published

Nayak, S.; Lee, H.; Chmielewski, J.; Lyon, L. A., "Folate-Mediated Cell Targeting and Cytotoxicity Using Thermoresponsive Microgels", *J. Am. Chem. Soc.*, vol. 126, (2004), p. 10258. Published

Book(s) of other one-time publications(s):

Jones, C. D.; Lyon, L. A., "Core/Shell Hydrogel Nanoparticles" , bibl. Marcel Dekker, NY; 5 Volumes, 3200 Pages; ISBN: 0-8247-4797-6, (2004). *Book* Published
of Collection: James A. Schwarz, Dr. Cristian Contescu, Dr. Karol Putyera, "Encyclopedia of Nanoscience and Nanotechnology"

Other Specific Products:

Contributions:

Contributions within Discipline:

Within the discipline of polymer science, we have extended the knowledge and understanding of how core/shell type hydrogel particles behave under a variety of conditions. We have also extended the range of synthetic methodologies that can be applied to the construction of stimuli-sensitive nanomaterials. This includes the construction of hollow particles that may be excellent surrogates for traditional liposomal methods of drug delivery. Our work on PEG-modified particles opens up new opportunities for the development of materials with well-controlled resistance to protein adsorption. The fundamental studies on core compression and restriction have allowed us to better understand how specific synthetic methodologies can control the mechanical and thermodynamic behaviors of hydrogel particles. We are also developing particles with well-defined pore structures that may have utility in bioanalysis or biocatalysis applications. Finally, our creation of targeted, triggered, non-pharmacophore anti-tumor particles may have broad impact in how particulate materials impact future cancer treatments.

Contributions to Other Disciplines:

We expect that our work on responsive nanomaterials will contribute to the fields of chemical and biological sensing and drug delivery. Indeed, others are using similar materials for such applications. Our work has extended the range of synthetic approaches to such particles and will hopefully impact how others design their hydrogels for specific applications.

Contributions to Education and Human Resources:

One former student (Dr. Clint Jones, Assistant Professor at Mercyhurst College) and a postdoc (Dr. Daoji Gan, E&A, Inc.) contributed to the early work on this project. They were followed by, four graduate students: Dr. Satish Nayak (Postdoc at University of Chicago), Bart Blackburn (current 4th year student), Jonathan McGrath (current 5th year student), and Neetu Singh (current 4th year student). Undergraduates Christina Baker (female Latino student) and Robert Bibb have also been involved in these studies. Christina Baker was awarded a GT Summer Research Fellowship for her work on this project; she is currently in the chemistry Ph.D. program at UCLA. Kristin Shepherd (African-American woman, Lincoln University) participated in this work through the GT NSF-REU program.

Contributions to Resources for Research and Education:

Our work on energy transfer probes of hydrogel phase transitions has provided a tangible route to introducing the complex process of energy transfer into the classroom. Dr. Lyon has used results from his own lab in the instruction of Analytical Spectroscopy to graduate students. This is an accessible and fundamental example of the technique. Many other literature examples involve complex biological systems, where many of the students lack sufficient background for good understanding. By first introducing the concept with structurally simple synthetic polymers, an understanding of the more complex systems becomes more trivial.

Categories for which nothing is reported:**Research and Education Activities****Findings****Products: Other Specific Product****Products: Internet Dissemination****Contributions Beyond Science and Engineering**

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Research Activities

As described in the original grant proposal, the overarching goals of this work were to understand the fundamental structure/function relationships in core/shell syntheses, and to create functional, multiresponsive particles from such constructs. Each the activities related to each subproject are described below.

Core/Shell Fundamentals/Core Compression

Jonathan McGrath continued the work of Dr. Clint Jones on using fluorescent probes to understand core compression following shell addition. He performed detailed FRET studies, fluorescence anisotropy, and fluorescence lifetime measurements on particles as a function of shell thickness, density, and dye loading.

Photo-triggered Particles

Satish Nayak has developed particles containing temperature jump dyes such as malachite green in order to create photo-responsive particles. He has investigated the photothermal properties of these particles as a function of dye loading and measurement conditions.

Hollow Shells (Degradable Cores)

We have continued our work on core/shell particles containing a cleavable diol crosslinker (*N,N'*-(1, 2-dihydroxyethylene)bisacrylamide, DHEBA) in the core. Satish succeeded in synthesizing particles that contain chemically cleavable sites for the purposes of creating hollow particles and sieving shells.

Sieving Particles

We have been able to use the cleavable diol chemistry to synthesize a series of particles with well-controlled and tunable pore sizes in the shell. By localizing the ligand biotin in a particle core and then coating that particle with a dense, degradable shell, Satish has been able to investigate the ability of avidin (the natural binding partner for biotin) to penetrate the shell as a function of diol cleavage.

Cellular Targeting

Satish has synthesized thermoresponsive particles with surface-localized amines, which are then used for coupling to the vitamin folic acid. In collaboration with Jean Chmielewski (Purdue University) these particle have been evaluated for their ability to enter cancer cells by receptor-mediated pathways. We have also investigated the cytotoxicity and endosomal escape of such particles.

Human Resources

One former student (Dr. Clint Jones, Assistant Professor at Mercyhurst College) and a postdoc (Dr. Daoji Gan, E&A, Inc.) contributed to the early work on this project. They were followed by, four graduate students: Dr. Satish Nayak (Postdoc at University of Chicago), Bart Blackburn (current 4th year student), Jonathan McGrath (current 5th year student), and Neetu Singh (current 4th year student). Undergraduates Christina Baker (female Latino student) and Robert Bibb have also been involved in theses studies. Christina Baker was awarded a GT Summer Research Fellowship for her work on this project; she is currently in the chemistry Ph.D. program at

UCLA. Kristin Shepherd (African-American woman, Lincoln University) participated in this work through the GT NSF-REU program.

Selected Presentations

“Stimuli Sensitive Colloidal Hydrogels”, *GRC on Chemical Sensors and Interfacial Design*, Salve Regina University, Newport, RI, August 5, 2003.

“FRET as a Probe of Core/Shell Microgel Structure”, *Inter-American Photochemical Society Winter Conference*, Clearwater, FL, January 3, 2003.

“Design and Assembly of Stimuli-Sensitive Colloidal Hydrogels”, *Organic/Inorganic Seminar* at University of Chicago, January 17, 2003.

“Design and Assembly of Responsive Hydrogel Nanoparticles”, *Analytical Division Seminar* at University of Wisconsin-Madison, September 19, 2002.

As part of this work, numerous student and postdoc oral presentations have been made at national meetings: Satish Nayak (2003 ACS (New York); 2003 ACS Colloid and Surface Science Symposium), Clint Jones (2002 ACS (Orlando); 2003 ACS Colloid and Surface Science Symposium), Daoji Gan (2002 ACS (Orlando)). Students also routinely attend and present at the Southeast Regional ACS Meeting.

Educational Activities

The graduate students working on this project have received an education in polymer and particle synthesis, electron and light microscopy, fluorescence techniques, organic synthesis (new monomers), light scattering methods, and NMR. Our FRET work has been used to introduce the concept (in part) to graduate students taking Dr. Lyon’s Analytical Spectroscopy course.

Research Findings

We were very successful in the grant period, as we accomplished or substantively addressed all of the main goals of the original project plan, and then pushed the work in directions that had not been previously considered. The original goals of this program related to the synthesis of higher-order core/shell hydrogel particles for potential applications in drug delivery and the detailed characterization of these topologically complex structures.

A major issue relates to how the hydrogel shell impacts the structure and responsivity of the core. Three manuscripts addressed the restriction of core swelling following shell addition by light scattering and fluorescent probes.¹⁻³ In these studies, we elucidated the role of shell addition in core behavior, wherein the added shell tends to “shrink-wrap” or restrict core swelling under certain conditions. This behavior arises from the inhomogeneous incorporation of cross-linker into microgels under our synthetic conditions. A particularly striking example of this effect is shown in Figure 1, which illustrates the use of fluorescence resonant

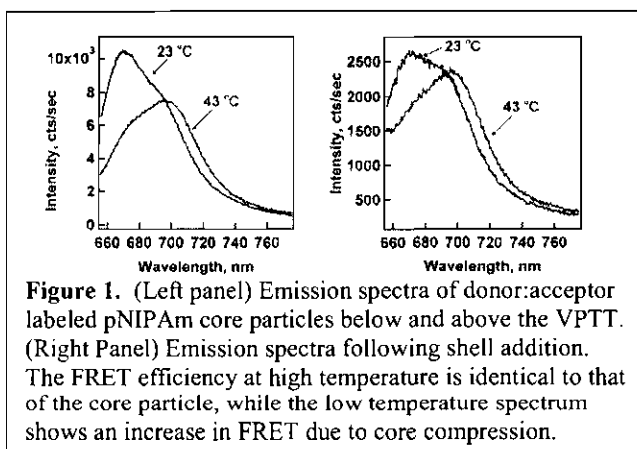


Figure 1. (Left panel) Emission spectra of donor:acceptor labeled pNIPAm core particles below and above the VPTT. (Right Panel) Emission spectra following shell addition. The FRET efficiency at high temperature is identical to that of the core particle, while the low temperature spectrum shows an increase in FRET due to core compression.

energy transfer (FRET) to quantify the effect. When the donor:acceptor pair Cy5: Cy5.5 is covalently incorporated into the core component of a pNIPAm particle, the energy transfer between the two dyes, is a direct measure of the inter-dye distance and hence the network volume. Upon shell addition, we observed a marked increase in energy transfer efficiency, which was evidence that the shell structure prohibited the core from swelling to the same volume it occupied prior to shell addition. Complementary studies using dynamic light scattering as a characterization technique have probed the influence of shell thickness and microgel cross-link density. In a related study, we measured the FRET from labeled core/shell particles to understand phase transition hysteresis.⁴ By covalently coupling the energy transfer donor (phenanthrene) to the core, followed by addition of an acceptor (anthracene) labeled shell, we were able to uniquely probe the core/shell interface. Interestingly, these studies showed a striking hysteresis in the phase transition behavior when it was probed with FRET, while turbidity and dynamic light scattering (DLS) measurements show no hysteresis. These results illustrate that while the swelling/deswelling transitions are reversible on the particle length-scale, probing the transition at shorter distances reveals that the swelling and deswelling *pathways* are in fact different.

The influence of poly(ethylene glycol) (PEG) grafts on phase transition behavior and protein adsorption onto microgels has been investigated.⁵ We used DLS, protein adsorption measurements, and ¹H NMR to understand the effect of PEG grafting on microgel behavior. A series of core/shell microgels was synthesized containing various amounts of PEG (1000 MW) monoacrylate as a macromonomer. As expected, increasing the PEG content in a simple “core” microgel decreased the degree of protein adsorption both below and above the volume phase

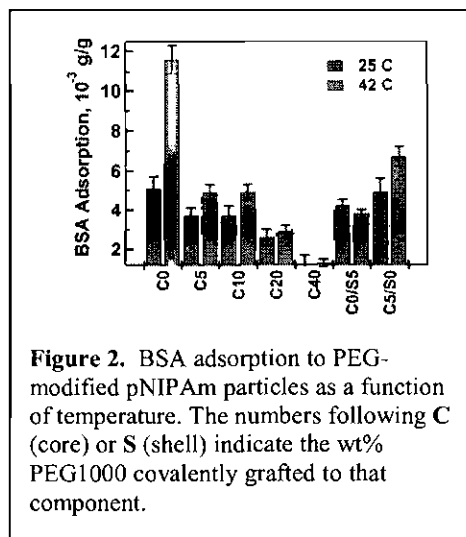


Figure 2. BSA adsorption to PEG-modified pNIPAm particles as a function of temperature. The numbers following C (core) or S (shell) indicate the wt% PEG1000 covalently grafted to that component.

transition temperature (VPTT). This was an important result with respect to the utility of pNIPAm in biomedical applications, as the deswollen homopolymer is hydrophobic and promotes protein aggregation. An unusual effect was observed, however, when the PEG was covalently localized in the core of a core/shell microgel, where the shell contained no PEG. We naively expected the particle to behave similarly to a simple pNIPAm particle, since the two particles should have similar outer microgel surfaces. However, as illustrated in Figure 2, this is not the case. The “core-buried” PEG is apparently still able to phase separate to the microgel surface upon particle deswelling, thereby reducing the particle surface energy. This hypothesis was further substantiated by NMR experiments. Below the VPTT, both the PEG and pNIPAm resonances are clearly resolved in the spectrum. However, above the VPTT the pNIPAm resonances become broadened and diminished in intensity as the polymer adopts a condensed conformation. The PEG resonances, on the other hand, remain sharp and well resolved suggesting those chains are in a solvent-rich environment, which may be evidence that the chains phase separate towards the surface to minimize the unfavorable mixing with deswollen pNIPAm.

Other studies have been undertaken to introduce different responsivities into microgels. For example, to modulate particle swelling via laser irradiation, we investigated the use of temperature-jump dyes to create photo-responsive nanogels.⁶ By coupling the dye malachite green isothiocyanate to amine-functionalized pNIPAm microgels, we were able to control particle swelling using HeNe laser irradiation ($\lambda=632.8$ nm). As expected, photoresponsivity was directly correlated to dye concentration. The photo-response exactly mirrored the behavior measured by bulk heating.

Another aspect of the original proposal involved the synthesis of hollow core/shell particles via degradable core polymers. A manuscript has recently been submitted on our work with well-controlled degradation chemistries for hollow shell fabrication.⁷ Core/shell particles were prepared wherein the core contained the cleavable cross-linker *N,N'*-(1,2-dihydroxyethylene)-bisacrylamide (DHEA). This cross-linker is stable under normal solution conditions, but can then be stoichiometrically cleaved through the addition of sodium periodate. Following core degradation, the remaining oligomers can be removed from the shell by dialysis, since they can freely diffuse through the shell pores. Particles such as these will remain a portion of the effort described below, as they have potential applications in controlled encapsulation and release of macromolecules.

Degradable cross-linker chemistries have also been employed to create pore-size tunable core/shell nanogels that sterically control protein binding. These are also useful materials in the study of protein diffusion in microgels.⁸ A highly complex core/shell microgel was constructed to illustrate the point. A pNIPAm-*co*-acrylic acid (pNIPAm-AAc) core was derivatized with the ligand biotin. These particles were then coated with ~35-nm thick pNIPAm shells that were cross-linked with various concentrations of DHEA. Avidin (60 kDa protein) binding to core-localized biotin is then dependent on diffusion of the protein through the shell network. As shown in Figure 3, at low DHEA concentrations (2 mol-%), avidin diffuses freely through the shell, as measured by the colorimetric 2-(4'-hydroxyazobenzene) benzoic acid (HABA) displacement assay. At high DHEA concentrations (20 mol-%), the network is much denser, and no avidin binding is observed. Controlled cleavage of DHEA by periodate results in no avidin binding until ~70% of the cross-links are degraded. However,

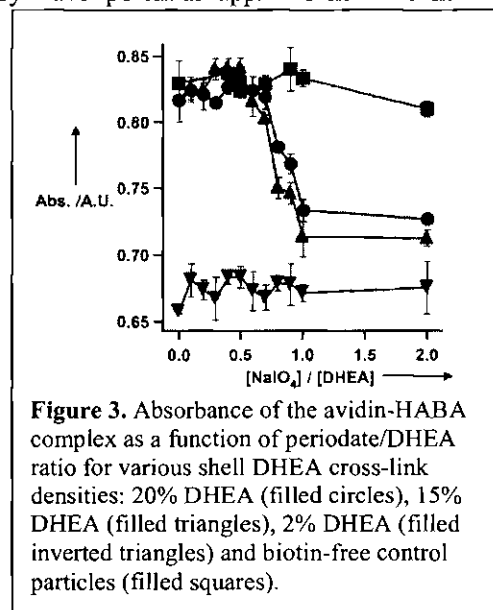
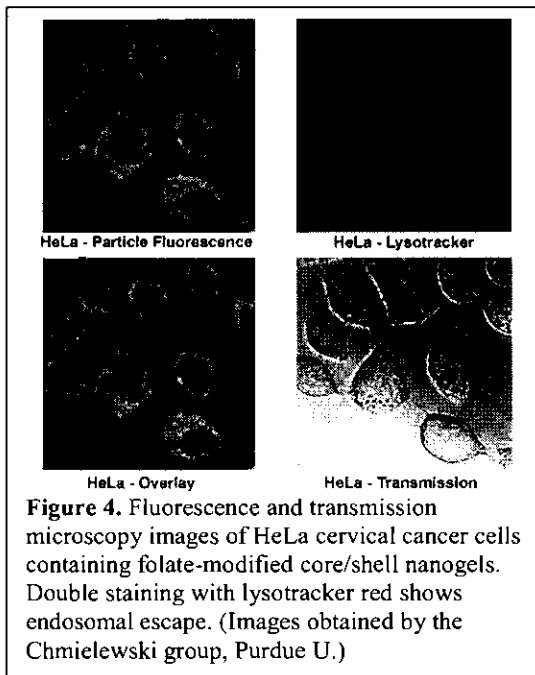


Figure 3. Absorbance of the avidin-HABA complex as a function of periodate/DHEA ratio for various shell DHEA cross-link densities: 20% DHEA (filled circles), 15% DHEA (filled triangles), 2% DHEA (filled inverted triangles) and biotin-free control particles (filled squares).

larger proteins, such as a 2:1 conjugate of horseradish peroxidase and avidin (150 kDa) is not able to

diffuse through the network under those conditions. We are continuing investigations of similar structures, which provide insight into protein diffusion as a function of particle density and core/shell topology.

Finally, we have tied together the design rules for core/shell nanogel synthesis to create nanogels capable of targeting cancer cells via receptor mediated endocytosis.⁹ PNIPAm and similar thermoresponsive polymers are of interest in drug delivery applications, as applying an external stimulus such as temperature can control the partitioning and expulsion of therapeutics such as anti-tumor agents. We synthesized pNIPAm core particles containing a small amount of 4-acrylamidofluorescein as a comonomer (for fluorescent tracking), to which a pNIPAm-co-*N*-(3-aminopropyl)methacrylamide hydrochloride (pNIPAm-APMA, 2 mol-% APMA) shell was added. The amine functionality was then used to covalently couple folic acid to the particle. Folic acid is an effective ligand for targeting tumors relative to healthy tissues. In collaboration with the group of Jean Chmielewski at Purdue University, these particles were tested for their ability to bind to and enter tumor cells overexpressing the folic acid receptor. Figure 4 shows some recent data from this collaboration, where confocal microscopy of HeLa cervical cancer cells shows excellent uptake of the particles. Furthermore, visualization of acidic compartments with the dye lysotracker red is used shows that many of the particles are able to escape from the endosomes and localize in the cytosol. This is an important feature of these particles, as endosomal entrapment of delivery vehicles dramatically decreases the therapeutic value of a treatment. This collaborative project is being continued; Profs. Chmielewski and Lyon have applied for funding through the NIH for this effort.



1. Jones, C. D.; Lyon, L. A., Characterization of Cyanine Dye-Labeled Poly(N-Isopropylacrylamide) Core/Shell Microgels using FRET. *J. Phys. Chem. B* **2004**, 108, (34), 12652-12657.
2. Jones, C. D.; Lyon, L. A., Dependence of Core Compression on Shell Thickness in Acrylic Acid Modified Poly(N-Isopropylacrylamide) Core/Shell Microgels. *Langmuir* **2003**, 19, (11), 4544-4547.
3. Jones, C. D.; Lyon, L. A., Shell-Restricted Swelling and Core Compression in Poly(N-Isopropylacrylamide) Core-Shell Microgels. *Macromolecules* **2003**, 36, 1988-1993.
4. Gan, D.; Lyon, L. A., Fluorescence Nonradiative Energy Transfer Analysis of Crosslinker Heterogeneity in Core-Shell Hydrogel Nanoparticles. *Anal. Chim. Acta* **2003**, 496, 53-63.
5. Gan, D.; Lyon, L. A., Synthesis and Protein Adsorption Resistance of PEG-Modified Poly-N-isopropylacrylamide Core/Shell Microgels. *Macromolecules* **2002**, 35, 9634-9639.
6. Nayak, S.; Lyon, L. A., Photoinduced Phase Transitions in Poly(N-isopropylacrylamide) Microgels. *Chem. Mater.* **2004**, 16, 2623-2627.
7. Nayak, S.; Gan, D.; Serpe, M. J.; Lyon, L. A., Hollow Thermoresponsive Microgels. *Small* **2005**, 1, (4), 416-421.
8. Nayak, S.; Lyon, L. A., Ligand-Functionalized Core/Shell Microgels with Permselective Shells. *Angew. Chem. Int. Ed.* **2004**, 43, 6706-6709.
9. Nayak, S.; Lee, H.; Chmielewski, J.; Lyon, L. A., Folate-Mediated Cell Targeting and Cytotoxicity Using Thermoresponsive Microgels. *J. Am. Chem. Soc.* **2004**, 126, (33), 10258-10259.