Pakistan-U.S. Science and Technology Cooperation Program
Final Report Form

Reports should be prepared jointly by the Pakistani and U.S. principal investigators and should cover all project-related activities carried out during the entire period on both sides. Please expand the boxes below to accommodate all the information you need to include.

Project Title: Synthesis and Characterization of Smart Polymer Microgels for Biomedical Applications	
Pakistani Principal Investigator: **Mohammad Siddiq**	**U.S. Principal Investigator:** **Shuiqin Zhou**
Reporting Period: 04/15/2008-07/31/2011	

Summary of Activities During Reporting Period: Please describe what has been accomplished under your grant during the reporting period, outlining the activities that were undertaken and specifically discussing achievements in relation to the milestones or targets set in the original work plan. For project-related events such as workshops, conferences, exchange visits, etc., please be sure to include exact dates and locations.

The objectives of the proposed project are (1) to synthesize and characterize a series of novel multiple responsive polymer microgel particles for biomedical applications and (2) to establish a polymer materials research lab equipped with a laser light scattering instrument in Quaid-I-Azam University. Our original work plan is listed as follows.

Year 1

• Selection and beginning of Pakistani graduate student to receive polymer synthesis training at the City Unviersity of New York (CUNY)

• Acquisition and installation of laser light scattering equipment and polymer synthetic facilities to establish a polymer materials research lab at QAU of Pakistan

• Selection of Research Associate and Initiation of research project at CUNY, including preliminary synthesis and data analysis of the proposed smart microgels

Year 2

• Training of Pakistani graduate student in physical characterization of polymers (functions of equipments, data acquisition, structure analysis, etc.) at CUNY

• Initiation of research project in the newly established polymer lab at QAU of Pakistan, including synthesis and detailed light scattering study of the proposed smart polymer particles

• More detailed morphology characterization of nanostructured microgels

• Present results in international conference and peer-reviewed journal

Year 3

• Permeability study of the smart microgel particles in response to external stimuli

• Loading and releasing behavior study of the novel smart microgels with different nanostructures for model protein drugs including Insulin

• Final report to HEC and report results in peer-reviewed journal

Our actual work followed our original work plan through the following outlined activities. All the objectives set for the project have been well accomplished during the

reporting period.

A. Recruiting and Training of Personnel

A.1. Selection of Research Associate and Initiation of Research Project at CUNY

Right after knowing the availability of this grant, we posted a job advertisement through CUNY research foundation to recruit a qualified research associate to work on our project. From the ten applications, we identified a research associate Dr. Weitai Wu to work on this project in US PI's lab. Dr. Wu's previous research experiences on smart polymeric materials perfectly fit our needs. During his stay from November 2008 to February 2011, Dr. Wu has not only worked very hard and outstandingly on this specific project, but also helped the US PI to train the graduate students and the PI from Pakistan PI's lab.

A.2. Selection and Training of Pakistani graduate student in polymer synthesis and physical characterization at CUNY

After the detail discussions through emails between the Pakistan and US PIs, Mr. Zahoor Hussain Farooqi, a second year graduate student at Pakistan PI's lab were selected and trained in US PI's lab. Following the new visa procedures for Pakistan-US Science and Technology Cooperative Program, we submitted all the required documents for Mr. Farooqi's visit to the US National Academies in middle June of 2008. The security risk and fraud inquiry interview for Mr. Farooqi was conducted by the USAID team at the end of August. However, the USAID team observed two problems and put on hold for Mr. Farooqi's visa application. One problem is that the applicant Mr. Farooqi shared his idea of a possible extension of the originally designed 15-month training at the US. Another problem is that the applicant was very weak in his spoken and listening skills of English. For the first issue, the PI in the US responsed timely to confirm that Mr. Farooqi's training will end exactly within the originally designed training length and no extension in this regard will be allowed. For the second issue, the US PI proposed Mr. Farooqi to attend an intensive English training course provided by the English Language Institute at College of Staten Island of CUNY (CSI-CUNY) at the start of his visit, with the days and times he is not attending English course to be devoted to his research training in the US PI's lab. The cost of the English course reduced the living stipends, so the duration of Mr. Farooqi's visit was reduced from 15 months to 13.5 months. At the beginning of September of 2008, Dr. Robbins at the NAS wrote an official letter to the USAID team of US Embassy at Islamabad to confirm our response and steps to solve the two problems. Dr. Robbins also approved the budget revision request from US PI to reflect the proposed change for Mr. Farooqi's visit. With this clarification, Mr. Farooqi's visa application was approved and his flight itinerary was arranged.

Mr. Farooqi eventually arrived at CSI-CUNY on February 1, 2009. He started his intensive English training course at the English Language Institute of CSI-CUNY on 02/02/2009. The course ran 4 days per week for 14 weeks. He devoted his Friday (no English classes) to get initial research training in the US PI's lab, including literature search and learning some basic synthetic skills for smart polymeric microgels. The English course was ended on 05/08/2009. Mr. Farooqi passed all the tests and got a certificate for the completion of the English course. Right after the English training, he started to work on the project in the US PI's research labs full time. Both the US PI and the Research Associate Dr. Weitai Wu supervised him to learn the synthesis and characterization of smart polymeric microgel particles. He has been taught to synthesize

Ag nanoparticles, pH-sensitive microgels, glucose-responsive microgels, and the Ag-polymer hybrid microgels. Although it took quite while for him to pick up the new synthetic skills and design new polymeric microgel systems, he could eventually independently synthesize routine pH-, temperature-, and glucose-sensitive polymer microgels after the training. He was trained to use the dynamic laser light scattering, photospectrometers, and electronic microscopes to characterize the synthesized polymeric microgels as well. He was also trained on the data and result analysis to draw scientific conclusions and how to draft and revise a manuscript for publications.

Mr. Farooqi returned to Pakistan on March 8, 2010. He was able to apply what he learned in the US PI's lab to train more students in the Pakistan's lab on the synthesis and characterization of smart polymeric microgel related materials.

A.3. Training of the Pakistan PI at CUNY

The major goal of this project is to establish a polymer materials research lab equipped with a laser light scattering instrument at Quaid-i-Azam University. Due to the delay of HEC funding, the acquisition and installation of laser light scattering equipment was somewhat delayed. After receiving his grant funds, Dr. Mohammad Siddiq worked very hard to purchase the laser light scattering equipment for his lab. While awaiting its delivery and installation, he made a 2-month visit to the US PI's lab at CUNY CSI beginning on June 17, 2009. During his stay in Dr. Zhou's lab, he received training on operation of the equipments, participated in joint research activities, and monitored his student Mr. Farooqi's research progress. Dr. Siddiq also received onsite installation training for the Laser Light Scattering spectrophotometer in the Brookhaven Instruments Corporation during his stay at CSI-CUNY (August 3-6, 2009). He also synthesized several series of smart polymer microgels that can undergo a volume phase transition in response to pH and temperature changes. Soon after Dr. Siddiq arrived home in August (he returned back to Islamabad on 08/14/09), his new equipment was installed and calibrated by a technician sent to Islamabad by the manufacturer. With the experience and skills he gained during his visit to CUNY, he was able to put the equipment to good use both in his own research and in educational activities for his students at Quaid-i-Azam University in Pakistan.

B. Establishing the Polymer Chemistry Laboratory for Synthesis and Characterization of Smart Polymer Microgels at QAU

After receiving the training at CUNY PI's lab, Dr. Siddiq and Mr. Farooqi were able to establish a new polymer materials research lab equipped with a new laser light scattering instrument at QAU with the funding support from HEC. Currently, the newly established polymer lab at QAU is very active in the synthesis and characterization of pH-, temperature-, and glucose-sensitive polymer microgels. After his return to QAU, Mr. Farooqi passed five Ph.D. courses. He also helped Dr. Siddiq to run the new polymer lab and train new graduate students. So far, Dr. Siddiq and Mr. Farooqi have trained six M. Phil students for synthesis and characterization of smart polymer microgel systems in the newly established Polymer Chemistry laboratory at QAU. The M.Phil students have been taught to synthesize Ag nanoparticles, pH-sensitive microgels, glucose-responsive microgels, and the Ag-polymer hybrid microgels. They were also trained to use the dynamic laser light scattering, photospectrometers to characterize the synthesized polymeric microgels. They have successfully synthesized and characterized several series of responsive microgels for biomedical and nanotechnological applications. The research

activities and findings in the newly established Polymer Research Lab at QAU are summarized as follows.

B.1. P(NIPAM-AAc) and P(NIPAM-AAm-AAc) Copolymer Microgels

We have prepared pH-sensitive and temperature responsive microgels based on N-isopropylacrylamide and acrylic acid. Different samples of multi-responsive copolymer, poly(N-isopropylacrylamide-co-acrylic acid) [P(NIPAM-co-AAc)] microgels were synthesized using different amounts of ingredients by precipitation polymerization. The prepared microgels were purified by dialysis for two weeks in order to remove unreacted monomers and surfactant. Dynamic Laser Light Scattering (DLS) was used to determine the diffusion coefficient and hydrodynamic radius (R_h) of the microgel at different pH and different temperatures. At pH = 7, the microgels showed rapid response to temperature and underwent volume phase transition (VPT) as we increased the temperature. At lower temperature, the size of microgel was large because the microgels were in hydrated or swollen state. At temperature above 38 ^oC, the size abruptly decreased because of the removal of water from the microgel. By increasing pH, the VPT temperature increased, but decreased on the addition of salt. Under different conditions of pH and NaCl concentrations, variation in VPT temperature value was observed. FTIR spectroscopy was used to confirm the chemical structures of microgels.

We have also successfully prepared pH-sensitive and temperature responsive microgels based on NIPAM, AAc, and acrylamide (AAm). Different samples of multi-responsive copolymer microgels of P(NIPAM-co-AAm-AAc) were synthesized using different amounts of ingredients by emulsion polymerization. The prepared microgels were purified by dialysis for two weeks in order to remove unreacted monomers and surfactant. Dynamic Laser Light Scattering (DLS) was used to determine the diffusion coefficient and hydrodynamic radius (R_h) of the microgel at different pH and different temperatures. FTIR spectroscopy was used to confirm the chemical structures of microgels.

B.2. Ag-P(NIPAM-AAc) and Ag-P(NIPAM-AAc-AAm) Hybrid microgels

We have successfully synthesized new sensing systems based on the immobilization of Ag nanoparticles in the multi-sensitive copolymer microgels of P(NIPAM-AAc) and P(NIPAm-co-AAm-AAc). The temperature and pH -induced volume phase transition of the polymer networks could modify the local refractive index around the Ag nanoparticles, resulting in sensitivity in UV-Vis absorption property. Thus we designed polymer microgel systems for tuning of optical properties of noble metal nanoparticles.

B.3. Glucose-responsive Microgels for Working at Physiological pH and Temperature

Although Mr. Farooqi has returned back to QAU at Pakistan, we maintain our collaborations through the email communications. Under the guidance of the US PI and Dr. Wu in US PI's lab, Mr. Zahoor Farooqi has learned how to synthesize and characterize the glucose-sensitive microgels for glucose sensing under physiological conditions. He continued his work on the synthesis and characterization of glucose-sensitive poly(N-isopropylacrylamide-4-vinylpyridine-phenylboronic acid) [p(NIPAM-4VP-PBA)] microgels with different percentage of 4-VP. The glucose-sensitive, pH responsive and thermosensitive behaviors of the microgels were investigated using dynamic light scattering. The feeding contents of the hydrophilic 4-VP group increase the hydrodynamic radius and volume phase transition temperature of the resultant microgels.

The effect of 4-VP contents on hydrodynamic radius and volume phase transition was systematically studied at different pH values. The effect of 4-VP contents on the glucose sensitivity was also investigated at physiological pH and temperature. The Lewis base moiety 4-VP adjusts the working pH of the PBA based glucose responsive microgels to physiological pH.

C. Design, Synthesis, and Investigation of Novel Smart Polymer Microgels in Different Compositions and Nanostructures for Biomedical Applications

We have successfully synthesized and investigated multiple series of hybrid responsive microgels for potential optical sensing of biological medium (including glucose monitoring), cancer cell imaging, and controlled drug delivery applications. The research activities and findings from US PI's lab are summarized as follows.

C.1. Tunable Photoluminescence of Ag Nanocrystals in Multiple-Sensitive Hybrid Microgels

We have successfully prepared fluorescent pH-sensitive hybrid materials based on the in situ immobilization of Ag and Ag/Au bimetallic nanoparticles (NPs) in the interior of multiple sensitive copolymer microgels of poly(*N*-isopropylacrylamide-co-acrylic acid-co-acrylamide) with polyacrylamide fragments serving as stabilizing ligands. Temperatures corresponding to the swollen, partially swollen, and nearly collapsed states of the template microgels were respectively applied for loading and in situ chemical reduction of Ag^+ ions to control the size and size distribution of the Ag NPs in the microgels. Au nanoclusters were stably grown onto the surface of Ag NPs by galvanic replacement reaction between Ag and $AuCl_4^-$. The pH-sensitive optical properties of hybrid microgels embedded with either Ag NPs or Ag/Au bimetallic NPs were systematically investigated. The gradual protonization of poly(acrylic acid) fragments shrank the microgels, resulting in an enhancement of photoluminescence (PL) intensity and a blue shift of the emission maximum of the Ag NPs. The surface modification of small Ag NPs with Au nanoclusters reduced the pH-sensitivity of PL property change, but dramatically enhanced the PL signals in the near infrared regime of the hybrid microgels. The method presented here opens a new door in the synthesis of materials with pH-responsive optical properties for biomedical applications.

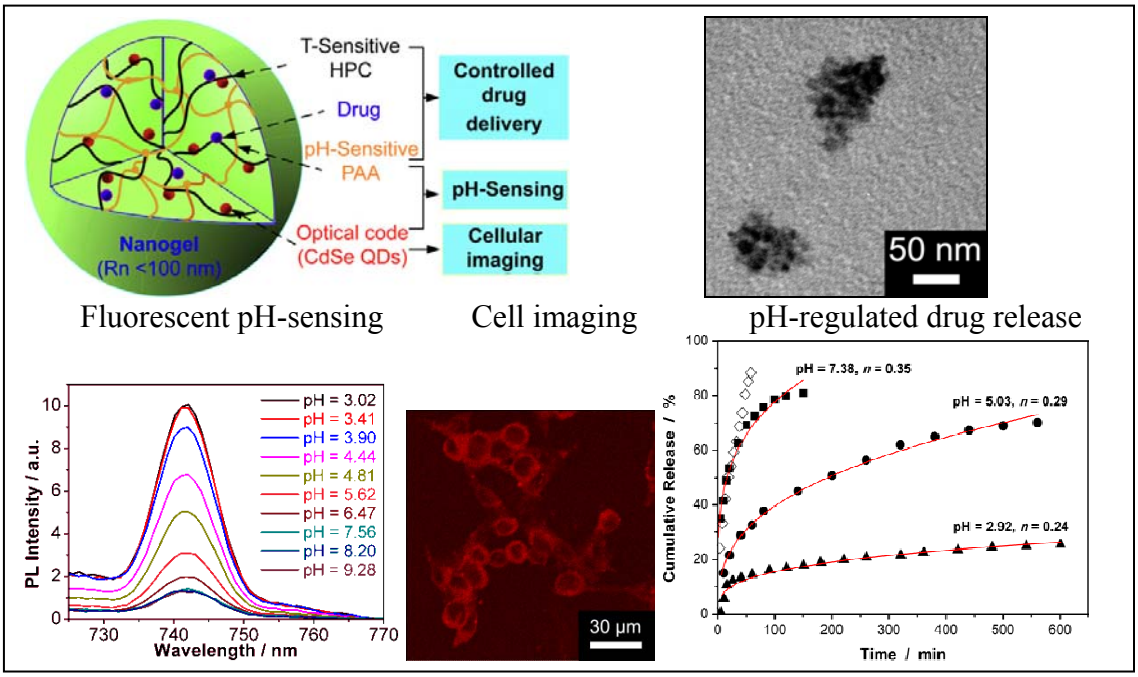
C.2. Optical Detection of Glucose by CdS Quantum Dots Immobilized in Smart Microgels

We have successfully synthesized new sensing systems based on the immobilization of fluorescent CdS QDs in the glucose-sensitive copolymer microgels of poly(*N*-isopropylacrylamide-co-acrylamide-phenylbronic acid) [p(NIPAM-AAm-PBA)]. The glucose-induced volume phase transition of the polymer networks could modify the local refractive index around the CdS QDs, resulting in glucose sensitivity in UV-vis property. The swelling of the microgels also affected the fluorescence features of the CdS QDs by the increase of the interfaced quenching centers, resulting in the quenching in PL intensity. The hybrid microgels can respond to glucose at physiologically important glucose concentration ranges, i.e., ≈2-25 mM. Thus, we developed a new recognition motif for glucose that shows promise for the fabrication of noninvasive or minimally invasive in vivo glucose sensing for patients with diabetes mellitus.

C.3. In-situ immobilization of quantum dots in polysaccharide-based nanogels for integration of optical pH-sensing, tumor cell imaging, and drug delivery

We have developed a class of multifunctional hybrid nanogels (R_h < 100 nm) through

the in-situ immobilization of CdSe QDs (~3.2 nm) into the hydroxypropyl cellulose-poly(acrylic acid) (HPC-PAA) semi-interpenetrating nanogels for integration of pH-sensing, tumor cellular imaging, and pH-regulated drug delivery. While the HPC chains can stabilize the CdSe QDs, the pH-sensitive PAA network chains can control the swelling degree of the nanogels, which can modify the surface physicochemical



properties of CdSe QDs for optical pH-sensing. The useful optical feature of the HPC-PAA-CdSe hybrid nanogels is that they emit at two different fluorescence wavelengths, combining a pH-sensitive NIR emission (741 nm) and a less sensitive visible emission (592 nm), which is beneficial for sensor and bioimaging applications. The hybrid nanogels can overcome cellular barriers to enter the intracellular region and light up the mouse melanoma B16F10 cells. The hybrid nanogels can also provide excellent stability as a drug carrier, which cannot only provide a high drug loading capacity of the model anticancer drug TMZ, but also offer pH-regulated drug delivery possibilities. The drug release kinetics studies demonstrate that the multifunctional nanogels show drug sustained properties and the release could be triggered by the pH-responsive volume phase transition of the gel. *In vitro* cytotoxicity tests indicate that the empty hybrid nanogels have very low cytotoxicity, while the TMZ-loaded hybrid nanogels have high anticancer activity. These characteristics are important in both fundamental research and practical applications. Stimulus-responsive hybrid nanogels, designed by integration of functional building blocks for sensing, imaging, and controlled drug delivery, offer broad opportunities for combined diagnosis and therapy.

C.4. Construction of optical glucose nanobiosensor with high sensitivity and selectivity at physiological pH on the basis of organic–inorganic hybrid microgels

We have developed a new class of optical glucose nanobiosensors with high sensitivity and selectivity at physiological pH. To construct these glucose nanobiosensors, the fluorescent CdS quantum dots (QDs), serving as the optical code, were incorporated into the glucose-sensitive poly(N-isopropylacrylamide-acrylamide-2-acrylamidomethyl-5-

fluorophenyl-boronic acid) copolymer microgels, via both in situ growth method and "breathing in" method, respectively. The polymeric gel can adapt to surrounding glucose concentrations, and regulate the fluorescence of the embedded QDs, converting biochemical signals into optical signals. The gradual swelling of the gel would lead to the quenching of the fluorescence at the elevated glucose concentrations. The hybrid microgels displayed high selectivity to glucose over the potential primary interferents of lactate and human serum albumin in the physiologically important glucose concentration range. The stability, reversibility, and sensitivity of the organic–inorganic hybrid microgel-based biosensors were also systematically studied. These general properties of our nanobiosensors are well tunable under appropriate tailor on the hybrid microgels, in particular, simply through the change in the crosslinking degree of the microgels. The optical glucose nanobiosensors based on the organic–inorganic hybrid microgels have shown the potential for a third generation fluorescent biosensor.

C.5. Chitosan-based responsive hybrid nanogels for integration of optical pH-sensing, tumor cell imaging and controlled drug delivery

We have successfully developed a new class of chitosan-based hybrid nanogels by in-situ immobilization of CdSe quantum dots (QDs) in the chitosan-poly(methacrylic acid) (chitosan-PMAA) networks. The covalently crosslinked hybrid nanogels with chitosan chains semi-interpenetrating in the crosslinked PMAA networks exhibit excellent colloidal and structural stability as well as reversible physical property change in response to a pH variation cross the physiological condition. In contrast, the hybrid nanogels formed by non-covalent physical association exhibit a significant change in the structure and composition upon exposure to physiological pH. This distinction in the structural stability of hybrid nanogels produces very different outcomes for their biomedical applications. The covalently crosslinked hybrid nanogels are low-cytotoxic and could illuminate the B16F10 cells, sense the environmental pH change, and regulate the release of anticancer drug in the typical abnormal pH range of 5e7.4 found in pathological zone, thus successfully combine multiple functionality into a single nano-object. However, the physically associated hybrid nanogels exhibit a non-reversible pH-sensitive PL property and a significant cytotoxicity after 24 h treatment. It is critical to construct a highly stable biopolymer-QD hybrid nanogel, via a rational design for safe bionanomaterials, to simultaneously combine the biosensing, bioimaging, and effective therapy functions. These characteristics are important in both fundamental research and practical applications.

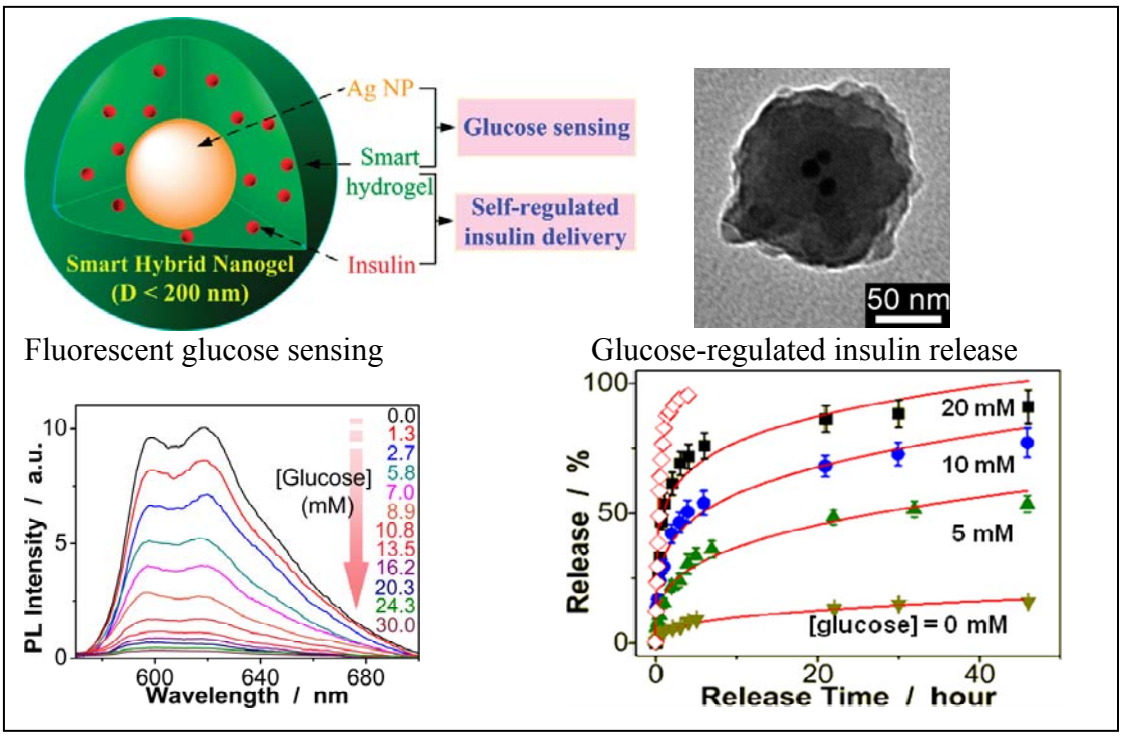
C.6. Smart Core-Shell Hybrid Nanogels with Ag Nanoparticle Core for Cancer Cell Imaging and Gel Shell for pH-Regulated Drug Delivery

Multifunctional nanoparticles that can provide long circulation and specific accumulation, illuminate the targeted object, and intelligently dose the pathological zones will enable major advancements in diagnosis and therapy. To facilitate a simultaneous tumor-cell imaging and adequate local delivery to the tumor site, we have developed core-shell structured hybrid nanogels (40-80 nm) composed of a Ag nanoparticle (NP) as core and smart gel of poly(N-isopropylacrylamide-co-acrylic acid) as shell. The pH-induced shrinkage of the nanogel increases the UV-vis absorption intensity and causes a blue shift of the surface plasmon bands of the Ag NP core. The smart nanogel can overcome cellular barriers to enter the intracellular region and light up the mouse melanoma B16F10 cells, including the nuclear regions. The surface property and

concentrations of the hybrid nanogels influences their interactions with cells, resulting in different cell morphology and selective cell staining. The pH-responsive hybrid nanogels exhibit not only a high drug loading capacity but also a pH-controllable drug releasing behavior. Smart hybrid nanogels with optical and therapeutic functionality, designed by integration of functional building blocks, offer broad opportunities for biomedical applications.

C.7. Multifunctional Hybrid Nanogel for Integration of Optical Glucose Sensing and Self-Regulated Insulin Release at Physiological pH

Optical detection of glucose, high drug loading capacity, and self-regulated drug delivery are simultaneously possible using a multifunctional hybrid nanogel particle under a rational design in a colloid chemistry method. We have developed a class of hybrid nanogels consisting of Ag nanoparticle (NP) cores covered by a copolymer gel shell of poly(4-vinylphenylboronic acid-*co*-2-(dimethylamino)ethyl acrylate) [p(VPBA_DMAEA)]. The introduction of the glucose sensitive p(VPBA_DMAEA) gel shell onto Ag NPs made the polymer-bound Ag NPs responsive to glucose. While the small sized Ag cores (10±3 nm) provided fluorescence as an optical code, the responsive polymer gel shell could adapt to a surrounding medium of different glucose concentrations over a clinically relevant range (0-30 mM), convert the disruptions in homeostasis of glucose level into optical signals, and regulate release of preloaded insulin. This shows a new proof-of-concept for diabetes treatment that exploits the properties from each building block of a multifunctional nano-object. The highly versatile multifunctional hybrid nanogels could potentially be used for simultaneous optical diagnosis, self-regulated therapy, and monitoring of the response to treatment.



C.8. Core-shell hybrid nanogels for integration of optical temperature-sensing, targeted tumor cell imaging, and combined chemo-photothermal treatment

We reported a class of core-shell structured hybrid nanogels to demonstrate the

conception of integrating the functional building blocks into a single nanoparticle system for simultaneously optical temperature sensing, cancer cell targeting, fluorescence imaging, and combined chemo-photothermal treatment. The hybrid nanogels were constructed by coating the Ag-Au bimetallic NP core with a thermo-responsive nonlinear poly(ethylene glycol) (PEG)-based hydrogel as shell, and semi-interpenetrating the targeting ligands of hyaluronic acid chains into the surface networks of gel shell. The Ag-Au NP core can emit strong visible fluorescence for imaging of mouse melanoma B16F10 cells. The reversible thermoresponsive volume phase transition of the nonlinear PEG-based gel shell cannot only modify the physicochemical environment of the Ag-Au NP core to manipulate the fluorescence intensity for sensing the environmental temperature change, but also provide a high loading capacity for a model anticancer drug temozolomide and offer a thermo-triggered drug release. The drug release can be induced by both the heat generated by external NIR irradiation and the temperature increase of local environmental media. The ability of the hybrid nanogels to combine the local specific chemotherapy with external NIR photothermal treatment significantly improves the therapeutic efficacy due to a synergistic effect.

C.9. Water-dispersible multifunctional hybrid nanogels for combined curcumin and photothermal therapy

We designed a class of water-dispersible hybrid nanogels for intracellular delivery of hydrophobic curcumin. The core-shell structured hybrid nanogels were synthesized by coating the Ag/Au bimetallic nanoparticles (NPs) with a hydrophobic polystyrene (PS) gel layer as inner shell, and a subsequent thin hydrophilic nonlinear poly(ethylene glycol) (PEG)-based gel layer as outer shell. The uniqueness of these hybrid nanogels lies in the integration of the functional building blocks for combined curcumin and photothermal therapy to significantly improve the therapeutic efficacy. The Ag/Au core NPs cannot only emit strong fluorescence for imaging and monitoring at the cellular level, but also exhibit strong absorption in the near-infrared (NIR) region for photothermal conversion. While the inner PS gel layer is introduced to provide strong hydrophobic interactions with curcumin for high drug loading yields, the external nontoxic and thermo-responsive PEG analog gel layer is designed to trigger the release of the pre-loaded curcumin either by variation of surrounding temperature or exogenous irradiation with NIR light. Such designed multifunctional hybrid nanogels are well suited for in vivo studies and clinical trials, thereby likely to bring this promising natural medicine of curcumin to the forefront of therapeutic agents for cancers and other diseases.

C.10. A Multifuntional Nanoplatform Based on Responsive Fluorescent Plasmonic ZnO-Au@PEG Hybrid Nanogels

Under a rational design, combining multiple constituents into a single nanoobject will not only bridge the unique properties of individual materials to leverage research both fundamentally and practically, but will also improve conventional sensing, imaging, and therapeutic effi cacies. We have successfully constructed such a nanoobject (< 100 nm) by covalently bonding ZnO quantum dots (QDs) to nonlinear poly(ethylene glycol)-based nanogel network chains, followed by appropriate growth of metallic Au. With the polymer gel network serving as a three-dimensional scaffold, the fluorescence of ZnO QDs could be well protected, while metal Au still retained its surface plasmon resonance property. The ZnO QDs covalently bonded to the thermo-responsive gel network chains can sensitively respond to temperature change of the surrounding fluids over the

physiologically important range of 37–42 ° C, converting the disruptions in homeostasis of local temperature into stable, robust and high-resolution fluorescent signals. The thermoresponsive hybrid nanogels can not only enter into and light up B16F10 cells, but also regulate the release of a model anticancer drug, temozolomide, in response to either local environmental temperature change or external near-infrared light-induced localized hyperthermia from metal Au. The combined chemophotothermal therapy can significantly improve the therapeutic efficacy due to a synergistic effect.

Educational Impacts: Please provide information on the numbers of students and other junior collaborators (graduate and undergraduate students, healthcare workers, laboratory technicians, data collectors, etc.) involved in the projects, and discuss new courses or degree programs created (if any) or changes to existing course curricula as a result of your project.

In the newly established polymer research lab at QAU (PI: Dr. Siddiq), one Ph.D. student (Mr Zahoor Hussain Farooqi) and six M. Phil graduate students (five male and one female) have participated this project and completed or nearly completed their Ph.D. or M. Phil degrees.

In the US PI's laboratory, in addition to the training of Mr. Farooqi and the Research Associate Dr. Weitai Wu, two female Ph.D. graduate students and two undergraduate students (one male and one female) have participated this project although they were not supported from this project. One female Ph.D. student is currently preparing her Ph.D. thesis defense. One undergraduate student graduated with B. S. degree in the summer of 2010 from CUNY-CSI. Other graduate and undergraduate students are continuing to work on the smart hybrid nanogels for biomedical applications.

Infrastructure Development: Please list any equipment acquired during this reporting period with grant funds and discuss the impact the new equipment will have on research and educational activities.

A Laser light-Scattering spectrophotometer was purchased from the Brookhaven instrument Corporation, New York. This new equipment funded by the Higher Education Commission (HEC) of Pakistan allows the Pakistan's PI Dr. Siddiq to establish a new polymer research lab for studying polymeric and colloidal materials. Currently, the Laser light-Scattering spectrophotometer is in full function at QAU. Dr. Siddiq is now able to put the equipment to good use both in his own researches and in educational activities for his graduate and undergraduate students.

Publications: Please provide citations for any papers published or conference presentations made as a result of your project.

At least a further couple of papers will be submitted by the end of 2011 about the multifunctional hybrid microgels for biomedical applications. The following list is a summary of what has been accomplished to date.

A. International peer-reviewed highly impact journals

1.Weitai Wu, Ting Zhou, Jing Shen, **Shuiqin Zhou "**Optical Detection of Glucose by CdS Quantum Dots Immobilized in Smart Microgels", ***Chem. Comm.*** **2009**, 4390-4392.

2. Weitai Wu, Ting Zhou, **Shuiqin Zhou** "Tunable Photoluminescence of Ag Nanocrystals in Multiple-Sensitive Hybrid Microgels", ***Chem. Mater.,*** **2009**, *21*, 2851-2861.

3. Weitai Wu, Michael Aiello, Ting Zhou, Alexandra Berliner, Probal Banerjee, **Shuiqin Zhou** "In-situ immobilization of quantum dots in polysaccharide-based nanogels for integration of optical pH-sensing, tumor cell imaging, and drug delivery", **Biomaterials**, **2010***, 31*, 3023-3031.

4. Weitai Wu, Jing Shen, Probal Banerjee, **Shuiqin Zhou** "Chitosan-based responsive hybrid nanogels for integration of optical pH-sensing, tumor cell imaging and controlled drug delivery", **Biomaterials, 2010,** *31*, 8371-8381.

5.Weitai Wu, Ting Zhou, Michael Aiello, **Shuiqin Zhou** "Construction of optical glucose nanobiosensor with high sensitivity and selectivity at physiological pH on the basis of organic-inorganic hybrid microgels", **Biosensors and Bioelectronics, 2010,** *25*, 2603-2610.

6. Weitai Wu, Ting Zhou, Alexandra Berliner, Probal Banerjee, **Shuiqin Zhou** "Smart Core-Shell Hybrid Nanogels with Ag Nanoparticle Core for Cancer Cell Imaging and Gel Shell for pH-Regulated Drug Delivery", **Chemistry of Materials**, **2010**, *22*, 1966-1976.

7. Weitai Wu, Nivedita Mitra, Elsa C. Y. Yan, **Shuiqin Zhou "**Multifunctional Hybrid Nanogel for Integration of Optical Glucose Sensing and Self-Regulated Insulin Release at Physiological pH", **ACS Nano, 2010**, *4* (8), 4831–4839.

8. Weitai Wu, Jing Shen, Probal Banerjee, **Shuiqin Zhou** "Core-shell hybrid nanogels for integration of optical temperature-sensing, targeted tumor cell imaging, and combined chemo-photothermal treatment", **Biomaterials, 2010,** *31,* 7555-7566.

9. Ting Zhou, Weitai Wu, **Shuiqin Zhou** "Engineering Oligo(ethylene glycol)-Based Thermosensitive Microgels for Drug Delivery Applications", **Polymer, 2010**, *51*, 3926-3933.

10. Weitai Wu and Shuiqin Zhou "Hybrid micro-/nanogels for optical sensing and intracellular imaging", **Nano Reviews, 2010**, 1: 5730 - DOI: 10.3402/nano.v1i0.5730.

11. Weitai Wu, Jing Shen, Probal Banerjee, **Shuiqin Zhou** "Water-dispersible multifunctional hybrid nanogels for combined curcumin and photothermal therapy", **Biomaterials, 2011,** *32*, 598-609.

12. Weitai Wu, Jing Shen, Probal Banerjee, **Shuiqin Zhou** "A multifunctional nanoplatform based on responsive fluorescent plasmonic ZnO-Au@PEG hybrid Nanogels", **Advanced Functional Materials, 2011**, *21*, 2830-2839.

13. Zahoor H. Farooqi, Weitai Wu, **Shuiqin Zhou**, Mohammad Siddiq "Engineering of Phenylboronic Acid Based Glucose-Sensitive Microgels with 4-Vinylpyridine for Working at Physiological pH and Temperature", **Macromolecular Chemistry and Physics**, **2011**, *212*, 1510-1514.

14. Zahoor H. Farooqi, Abbas Khan, Mohammad Siddiq "Temperature induced volume change and glucose sensitivity of poly[(Nisopropylacrylamide-co-acrylamide-co-(phenylboronic acid)] Microgels**", Polymer International, 2011, in press,** DOI:10.1002/pi.3106.

B. Papers presented at Conferences

1. Weitai Wu, Ting Zhou, Michael Aiello, Shuiqin Zhou. "Immobilization of quantum dots in multiple responsive microgels for biomedical applications". Proceeding of SPIE **2010**, in USA. (Paper ID: 7575-15) (Invited talk, presented by Shuiqin Zhou)

2. Weitai Wu, Shuiqin Zhou. "Immobilization of inorganic nanoparticles into smart polymer nanogels for integration of sensing, cell imaging, and controlled drug delivery". 240th ACS National Meeting, **2010**, in USA. (Paper ID: 15269) (Invited talk, presented by Weitai Wu)

3. Mohammad Siddiq, Invited talk. The workshop on Applications of Nanotechnology (WANT-2010) (31st May-04th June) organized by The Magnetic Nanostructures Group, Physics Department, Quaid-I-Azam University Islamabad.

4. Mohammad Siddiq, Invited talk. National Centre for Physics (NCP) with the support of US National Science Foundation (NSF) at National Centre for Physics, Islamabad Pakistan.

5. Zahoor Hussain Farooqi presented his research work on First National Conference on Physical and Environmental Chemistry (PEC-2010) organized by National Centre of Excellence in Physical Chemistry, The University of Peshawar Summer Campus, Bara Gali, Distt. Abbottabad 26th- 30th September 2010.

Additional Funding: Please list any additional funding applied for or received to help support your project during this reporting period.

The Pakistani PI received a small internal grant from University research fund (URF) to support partial research supplies for this project.

The US PI received two small internal grants from CUNY (PSC-CUNY research award) to support partial research supplies for this project.

Linkages with Government or Private Industry: Please describe any linkages developed with government agencies or companies interested in implementing the results of your project.

No.

Problems Encountered: Please provide information on any problems you may have encountered in making progress on your project objectives and describe steps you are taking to resolve the problems.

The only real problem encountered was the reduction and late release of funds from HEC to Dr. Siddiq. The acquisition and installation of laser light scattering equipment was somewhat delayed. However, Dr. Siddiq worked very hard to speed up the process. He negotiated the prices with Brookhaven Instruments Corporation to purchase the Laser Light Scattering (LLS) equipment for his lab before the fund was available. He submitted the quotation for LLS equipment to the purchasing office of his university right after he received the fund. Due to the budget cut of Pakistan HEC for the second year visit of Pakistan PI, Dr. Siddiq had requested the US PI to shift the budget originally set for his third year visit to US PI's lab to second year so that he could visit CUNY in the summer of 2009. Dr. Robbins approved this request. We worked together to submit all the documents required for Dr. Siddiq's visit to NAS to process his visa application timely. Eventually, Dr. Siddiq was able to visit CSI-CUNY and Brookhaven Instruments Corporation in the summer of 2009 and obtain the necessary training to set his new Polymer research Lab at QAU on time.

Plans for Future Activities: Please provide details on any follow-on activities during the next year,

including planned exchange visits, training events, and ongoing research efforts.

The results accomplished so far have demonstrated that our newly developed smart polymer-based microgels have great potential for biomedical applications in biosensing, cell imaging, and controlled drug delivery. However, further research effort is needed to optimize the size, composition and nanostructures of the hybrid microgels to enable the microgel particles being used in clinical trials. For example, how to design the hybrid microgels for continuous monitoring of blood glucose level and self-regulated insulin delivery, which could possibly realize the artificial pancreas for diabetic patients and lead to a breakthrough in the management and cure of diabetes.

Supplementary Information: If applicable, please attach copies of project-related workshop or conference agendas, course curricula developed, summaries of research data collected in the course of the project, or articles about the project appearing in newspapers, journals, or Web sites. **Please note that your report will be posted on the program Web site, so please do not include any data you do not wish to make publicly available at this point in your research.**

Indicators	**Reporting Period:**
1. Number of higher education partnerships between Pakistani and U.S. institutions (see note below)	1
2. Number of journal articles, technical reports, books, or book chapters (published or accepted for publication) resulting from your project during the reporting period	14
3. Number conference presentations resulting from your project during the reporting period	5
4. Number of training events (courses, workshops, seminars, conferences, stakeholders' meetings) conducted on your project during the reporting period	2
5. Total number of Pakistanis making exchange visits on your project during the reporting period	2
Number of women	
Number of men	2
6. Total number of Americans making exchange visits on your project during the reporting period	
Number of women	
Number of men	
7. Total number of exchange visits overall during the reporting period	2
8. Total number of Pakistani PhD students involved in the project	1
Number of women	
Number of men	1
9. Total number of American PhD students involved in the project	2
Number of women	2
Number of men	
10. Total number of all other Pakistanis not listed above who participated in your project during the reporting period (Include in this	6

total those who were involved as researchers, MS or undergraduate students, technicians, or data collectors, as well as those who received formal training in workshops or courses or participated in conferences or stakeholders' meetings organized as part of the project.)	
Number of women	1
Number of men	5
11. Total number of all other Americans not listed above who participated in your project during the reporting period (Include in this total those who were involved as researchers, MS or undergraduate students, technicians, or data collectors, as well as those who received formal training in workshops or courses or participated in conferences or stakeholders' meetings organized as part of the project.)	4
Number of women	2
Number of men	2

Note on Question 1: For the number of higher education partnerships, please count the partnership between your institution and your Pakistani counterpart's institution as one. If your project also involves collaboration with other Pakistani institutions / US institutions (universities, research institutes, government agencies, or non-governmental organizations), please add each such additional institution to your total.