**Project Name:** Protein Purification Using Magnetic Nanoparticles

**Professor Daniel I.C. Wang, Chemical Engineering**

**Project Overview:**
The goal of this project is to use magnetic nanoparticles to affect the purification of monoclonal antibodies from animal cell culture broths. The studies will involve the synthesis and characterization of the magnetic nanoparticles. In addition, the attachment of Protein A onto the magnetic nanoparticles will be examined. Lastly, the adsorption and elution of the monoclonal antibodies from the HGMS will be examined.

**Faculty Involved:**
Professor Daniel I.C. Wang, Department of Chemical Engineering, MIT. Professors Joao Crespo and Professor Cecelia Roque (Department of Chemistry, Faculdade de Ciencias e Tecnologia, Universidade da Caparica, Universidade Nova de Lisboa).

**Ph.D. students involved:**
At MIT, I have assigned a Visiting Scientist, Mr. Kenji Takahashi on this project. In Portugal, the Ph.D. student has not yet been assigned since this project was initiated in November 2007.

**Expected Deliverables:**
The anticipated deliverables from this project will be:
• Excellent doctoral theses at MIT and Portugal
• Publications in high quality international journals
• Presentation at international and national conferences
• Establish Institutions in Portugal and MIT as the premiere organizations in new and novel protein purification technologies
• Filing of patents internationally
• License technology to appropriate companies.

**Results:**
This project was only started in November 2008. At MIT, we have started to investigate the following areas.

**Develop ability to tune surface charge and hydrophobicity of magnetic fluid coatings.** This will be done, whenever possible, with commercially available polymers, or polymers that can easily be synthesized in large quantities in an industrial setting. Surface charge and hydrophobicity affect both protein separation behavior and magnetic fluid stability.
**Investigate the effects of polymer molecular weight on clustering.** The particle size has a strong affect on the separation efficiency and protein capacity.
The ability to control cluster size would allow optimization for different

applications, which should greatly increase the industrial importance of the particles.
In Professor Cecelia Roque's laboratory in Portugal, she has proposed to initiate research in the following areas.

**Attachment of Protein A onto Magnetic Nanoparticles.** It is well accepted in the biotechnology industry the use of Protein A as an affinity ligand to bind monoclonal antibodies as the first step in the purification process. Protein A can be purchased from commercial biochemical reagent companies, in its native and recombinant forms. Similar to what has been successful, the binding of Protein A will be tested using ion-exchange moieties on the magnetic nanoparticles.

**Examination on the Binding Efficiency on Magnetic Nanoparticles.** The efficiencies on the binding of Protein A onto the magnetic nanoparticles will first be performed in small-scale laboratory studies. Aspects to study will include: protein stability over time and CIP treatment procedures; Protein A leaching under operating conditions; binding capacity of the magnetic adsorbent. The best ion-exchange moiety will then be used for monoclonal antibody purification.

**Affinity Adsorption of Monoclonal Antibody to Protein A on Magnetic Nanoparticle.** The Protein A-magnetic adsorbent will then be used on the purification of an anti-fibrin monoclonal antibody produced by Hybridoma cell (CRL-1606). Studies on the adsorption of the functionalized magnetic nanoparticles will include such parameters as pH, temperature, ionic strength using various salts. In addition, the kinetics of monoclonal antibody adsorption will also be studied, as well as the affinity and specificity of the magnetic adsorbent system, considering the complexity of the crude sample applied. The anti-fibrin antibody is selected since at MIT we have had a great deal of prior experience in the production of this antibody using the animal cell system. These studies will be conducted as batch purifications using small scale stirred reactors or other vessels.

**Timeline ((Through August 2008)**
The timeline for the research through August 2008 are:
• Select an appropriate Ph.D. candidate at UNL for this project.
• Determine the synthesis of the magnetic nanoparticles most appropriate chemistry for the coating of the magnetic nanoparticles. This will be achieved through a collaborative effort between MIT and UNL.
• Initiate the chemistry of protein A binding to the magnetic nanoparticles. This will be conducted collaboratively between MIT and UNL.