IREE: New Generation Tailored Adsorptive Membranes

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ABSTRACT

An IREE supplement to the main NSF grant titled New Generation Tailored Adsorptive Membranes was obtained in order to send two PhD students, one from Clemson University and one from Colorado State University, to Germany. The German host is Prof. Mathais Ulbricht, Professor of Technical Chemistry at the University of Duisburg-Essen in Essen. The first student, from Clemson University, spent three months in Germany from October 2007 to January 2008. In addition, the Principal Investigators, Profs. Wickramasinghe and Husson spent a week at the University of Duisburg-Essen reviewing the student's accomplishments as well as developing further collaborations. A manuscript is currently being prepared for submission to the Journal of Membrane Science, based on the results of the first student's visit. The second student from Colorado State University will spend 3 months in Professor Ulbricht's laboratory later this year. Profs. Wickramasinghe and Husson will make a second visit to the University of Duisburg-Essen when the second student is in Germany. NSF IREE funding has led to the submission of a proposal that aims to develop collaborative degrees between the University of Duisburg-Essen and Colorado State University and Clemson University.

INTRODUCTION

This NSF project is a multidisciplinary research collaboration between the Departments of Chemical and Biological Engineering at Colorado State University (CSU, awardee institution) and Chemical and Biomolecular at Clemson University (CU). We are developing adsorptive membranes as a new, high efficiency unit operation for the recovery and purification of the products of modern molecular biology, such as plasmid DNA and large proteins, as well as viruses and viral vectors. Carefully designed, high-throughput membranes are being developed using modern tools of surface engineering and modeled for use as alternative chromatographic supports to overcome the many limitations of chromatographic resins that are used typically for these separations.

Our host laboratory for the IREE project is that of Prof. Dr. Mathias Ulbricht at the University of Duisburg-Essen (UDE), Essen, Germany. Prof. Ulbricht has significant experience using free radical based polymerization to grow polymer brushes from the surface of various membranes. His laboratory is well equipped to conduct experiments on membrane modification and characterization, and especially performance testing.

The reasons for international collaboration carried out under IREE include

- providing two graduate students with unique overseas research experiences on a project that is challenging and relevant to their research in the USA
- building upon an existing international collaboration with a research group that has complementary expertise to ours
- utilizing research equipment not currently available at CSU or CU
- impacting the institutional goals at CSU and CU of developing overseas research opportunities for graduates in engineering
- helping to establish future research collaborations with UDE

Two PhD graduate students are working on the project, Nripen Singh from CU and Shytyug Loh from CSU. Nripen spent 3 months at UDE from Oct. 2006 – Jan. 2007. Shytyug will spend 3 months at UDE later this year.

The anticipated research and education outcomes include the following

- We will generate data on membranes that have been surface modified using two different methods. The membranes will be fully characterized. We will be able to compare performance for adsorption and elution of a simple protein, and we will determine actual binding patterns as a function of chemical modification method. These results will form the basis for collaborative publications and presentations.
- The two students will gain an international perspective on engineering research and educational methods.
- The CSU strategic plan calls for the establishment of international research opportunities for students. CU's goal is to increase activity in international programs. The German government is encouraging US undergraduates to conduct summer research in Germany. Prof. Ulbricht is very keen to develop a program where students from CSU and CU can conduct summer research at UDE. The proposed work will strengthen existing partnerships to achieve all of these goals.

RESEARCH ACTIVITIES AND ACCOMPLISHMENTS OF THE INTERNATIONAL COOPERATION

Program of Research Carried Out During International Research Experience

The research goal of this international experience was to demonstrate a method for preparing high-capacity adsorptive membranes by growing surface-tethered, charged polymer nanolayers from the surfaces of regenerated cellulose (RC) membranes (average pore diameter 1 µm). Surface-initiated atom-transfer radical polymerization was used to grow nanolayers of poly(acrylic acid) (PAA) from the membrane pore surfaces using a range of polymerization times. Characterization was done by ATR-FTIR to follow the modification steps. Water flux measurements illustrated that polymerization time could be used to controllably decrease average pore sizes. Pore size distributions measured by liquid dewetting permoporometry also illustrate the effect of growing polymer chains from the surface of the membrane pores. Static and dynamic binding capacities were measured for lysozyme on the PAA-modified RC membranes. As shown in Table 1, capacities initially increase with increasing modification times and reach maximum values of 98.5 mg/mL (static) and 71.2 mg/mL (dynamic). These values are roughly three times higher than commercial ion-exchange membranes. Confocal laser scanning microscopy (CLSM) was used to visualize the protein binding of fluorescently labeled lysozyme on the unmodified and PAA-modified membranes. CLSM of labeled membranes (Figure 1) also showed that the modification procedure had no detrimental effects on the membrane pore structure.

Membrane modification time (min)	Static Capacity (mg/ml)	Dynamic Capacity (mg/ml)
Unmodified	0.0	0.0
30	50.2	47.0
60	98.5	71.2
90	75.8	44.8
180	79.9	54.3

Table 1. Membrane capacities.

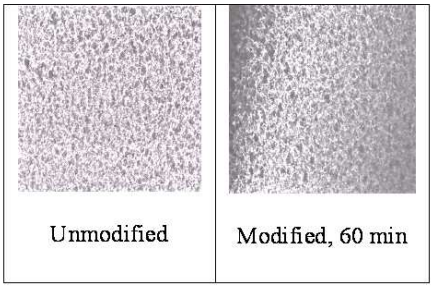

Figure 1. CLSM of labeled membranes.

Relationship Between the Work On-site and the Work of the Current NSF Award

The research program of the main NSF award aims to develop adsorptive membranes for the recovery and purification of the products of modern molecular microbiology. Ion-exchange membranes are being developed by functionalizing commercially available polymeric microporous membranes. The capacities of the membranes for capture of FDA recommended model viruses are being determined. The research aims of the IREE supplement are to compare the capacity of surface modified membranes for model protein and to use confocal microscopy to determine protein binding patterns. The membranes are modified by growing polymer brushes from the surface using two different methods, ATRP and conventional free radical polymerization.

Description of the General Interaction Between Researcher and Host Laboratory

During the research visit to Germany, Nripen had the opportunity to exchange scientific

knowledge and techniques with the host research group under the supervision of Prof. Ulbricht. Findings were discussed with Prof. Ulbricht at least once a week. Short meetings were also held on a regular basis with other students as well as one-on-one with Prof. Ulbricht whenever needed. During the course of laboratory experiments and research meetings, it was very helpful to share research and scientific experiences with the group of German students. Nripen provided feedback and suggestions on their common ATRP research problems. In return, he was trained on specialized equipment by the laboratory technician and students. Nripen presented his PhD work in detail through a formal talk/presentation at UDE, and he is currently working on a collaborative manuscript to publish the findings of the research project conducted in Germany.
Nripen also interacted socially with the group and the community by visiting several places in Germany and participating in the Christmas event organized by Prof. Ulbricht. Social interactions helped Nripen to establish strong friendships with members of the research team and also increased his familiarity with the new cultural environment. Thus the research visit to Germany was an enriching experience for Nripen academically, socially, and personally, and he expresses his thankfulness to NSF and the host research group for the opportunity to have this learning experience.

IREE Project Schedule
The IREE project involves two PhD graduate students. The first student, Nripen Singh from CU, spent 3 months (Oct. 2006-Jan. 2007) in Prof. Ulbricht's lab at UDE to perform the membrane modification and characterization work using ATRP. The second student, Shytyug Loh from CSU, will spend 3 months later this year in Prof. Ulbricht's laboratory to perform modification and characterization work using free radical polymerization.

BROADER IMPACTS OF THE INTERNATIONAL TRAVEL

The original scope of the current award included using ATRP for all of the membrane modification work. ATRP is a promising new method for grafting polymer brushes from the surface of polymeric membranes; however, from a commercial perspective, ATRP remains an immature technology compared to standard free radical polymerization. Since standard free radical polymerization is not a living polymerization, it is far less controllable; however, it is a much older technology for which there is significant industrial experience. Thus, there are two main research aims for the IREE project that expand the original scope of the current award: To compare the effectiveness for capture of model proteins by polymer brushes grown using ATRP and free radical polymerization and to use confocal microscopy to determine protein binding patterns. By expanding the scope of the original proposal in this way, the results obtained as a consequence of IREE supplemental funding may lead to numerous fundamental and applied extensions of the work.
Prior to this IREE supplement, Prof Wickramasinghe spent 8 months (May 2004 – January 2005) in Germany while on sabbatical leave. During this period, he worked with Prof Ulbricht on the modification of cellulosic membranes by growing polymer brushes from the surface of the membranes, which were provided by Sartorius AG (Göttingen, Germany). In addition, Prof Wickramasinghe collaborated with the Forschungszentrum Jülich, Germany, to develop confocal microscopy as a technique to visualize binding

patterns of model proteins to ion exchange membranes [1]. The IREE supplement has provided an opportunity to build on this existing collaboration and to establish new collaboration between Clemson and UDE. Profs. Wickramasinghe and Husson spent 2 weeks in Germany as part of this program, during which they met with a number of researchers at UDE to discuss opportunities for further collaboration. A full proposal to the NSF PIRE program which includes the development of a collaborative PhD degree between the UDE and CSU and UDE and CU has been submitted.

DISCUSSION AND SUMMARY

Nripen Singh's visit to Germany has strengthened the research collaborations between the UDE and CSU and CU. As a consequence of his visit, we now know that ATRP may be used to make adsorptive ion exchange membranes. Further the capacities of these membranes for model proteins is significantly higher than commercially available ion exchange membranes. Thus ATRP could be a valuable new technology for manufacturing high capacity ion-exchange membranes. These results will be submitted to the Journal of Membrane Science. A second student from CSU will continue this work later this year. In addition, Nripen has benefited greatly from the opportunity to live and work in Germany.

Another major impact of IREE funding has been the submission of a full proposal to the NSF PIRE program. This proposal aims to develop collaborative degrees between UDE and CSU and UDE and CU. Thus IREE funding has helped to build and strengthen existing research collaborations established by Prof. Wickramasinghe during his sabbatical leave.

We believe the IREE program is an excellent mechanism by which graduate students and their advisors can build new international collaborations. In our case, the fact that Prof. Wickramsinghe, PI of the main NSF grant, had existing collaborations with Prof Husson and Prof. Ulbricht has ensured the successful outcome of IREE funding. Thus we believe that IREE supplements will have the biggest impact when the PIs have previous collaborations with the host institution. IREE funding will help build and extend these collaborations.

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BRIEF BIOGRAPHIES OF RESEARCHERS

Ranil Wickramasinghe received his Bachelor's and Master's degrees in Chemical Engineering from the University of Melbourne, Australia in 1986 and 1988. He received his Ph.D. in Chemical Engineering from the University of Minnesota in 1992. From 1992-1997 Prof Wickramasinghe worked in the Biotechnology industry for Separacor Inc. and Biogen Inc. both in the Boston area. Prof Wickramasinghe has been a faculty member in the Department of Chemical and Biological Engineering at Colorado State University since 1998. He currently holds the positions of Associate Professor and Associate Department head for graduate Studies. His research interests include development of new membrane based separation processes especially for bioseparations, biomedical separation and environmental separations

Scott Husson received the B.S. degree in Chemical Engineering from the Pennsylvania State University in 1993. He received his Ph.D. degree in Chemical Engineering from the University of California, Berkeley in 1998. Prof. Husson has been a faculty member in the Department of Chemical and Biomolecular Engineering at Clemson University since 1998. He currently holds the positions of Associate Professor and Undergraduate Coordinator, and he is acting Topic Leader in the NSF Center for Advanced Engineering Fibers and Films. Research interests include surface engineering by self-assembly and surface-initiated polymerization, bioseparation materials synthesis, molecular imprinting, and surface plasmon resonance spectroscopy.

Nripen Singh received his B.E. degree in Chemical Engineering from Panjab University in 2002. He will complete his Ph.D. in Chemical Engineering from Clemson University in June 2007. In 2006, he was named the Clemson University College of Engineering and Science Outstanding Graduate Researcher. Mr. Singh was a Visiting Scientist at the University of Duisburg-Essen from Oct. 2006-Jan. 2007 as part of this IREE grant. Research interests include polymer-peptide interactions, surface-initiated polymerization, and membrane modification.