Inverted Colloidal Crystal Membranes

Scott M. Husson[1], S. Ranil Wickramasinghe[2], Xianghong Qian[2], Ruben Kemmerlin[1], and Makenna Nielsen[2]
[1]*Clemson University, Department of Chemical and Biomolecular Engineering, Clemson, SC 29634, USA. E-mail: shusson@clemson.edu*
[2]*Colorado State University, Department of Chemical and Biological Engineering, Fort Collins, CO 80523, USA. E-mail: wickram@engr.colostate.edu*

ABSTRACT: An IREE supplement was obtained to the main NSF grant titled Inverted Colloidal Crystal Membranes in order to provide international research experiences to two undergraduate students, one from Clemson University (CU) and one from Colorado State University (CSU). The primary rationale for this IREE supplement was to test run a new collaborative undergraduate program between CU and CSU. I-PRIME (Incubator Program for Research with International Mentors and Educators) 'primes' undergraduates for international research. In this program, the undergraduates participated for one year in existing research programs at CU or CSU to 'PRIME' them for research abroad. The summer after their primer year of research, they conducted research in Germany. The German host was Prof. Andreas Seidel-Morgenstern, Director and Head of the Physical and Chemical Foundations of Process Engineering Research Group at Max Planck Institute (MPI) of Dynamics of Complex Technical Systems and Chair, Chemical Process Engineering at Otto-von-Guericke-Universität, Magdeburg, Germany. The students, Ruben Kemmerlin and Makenna Nielsen, spent 3 months at the MPI from May – July 2008. In addition, one early career Assistant Professor, Xianghong Qian, spent 3 weeks at MPI. Profs. Husson and Wickramasinghe spent 1 week at the MPI to initiate the student projects and to develop plans for further collaborations. The objectives of the IREE program were to 1) Prepare students for professional employment and graduate education in the global workplace through I-PRIME, 2) Build strong international partnerships that enhance the research and education enterprises of all partners as a result of unique capabilities, 3) Prepare purified human influenza virus using a sequence of pseudo affinity chromatography steps 4) Extend the application potential of ICC membranes by modifying their surfaces with poly(ionic liquids) and measuring adsorption isotherms of carbon dioxide on the membranes, 5) Begin to investigate the conformational structures of modified membrane surfaces and their potential impact on binding and diffusion properties using molecular dynamics simulations. Objective 5 was specific to the work of the early career Assistant Professor, Prof. Qian. All of these objectives were complementary to the objectives of the main program. A manuscript is currently being prepared for submission based on the results of Objective 4. Another outcome of NSF IREE funding will be the submission of a proposal that aims to develop collaborative degrees between Otto-von-Guericke-Universität and Clemson University and Colorado State University.

INTRODUCTION

This NSF project is a multi-university, multi-disciplinary collaboration among Profs. Husson (PI, Chemical Engineering) and Benson (co-PI, School of Engineering Education, outreach) at Clemson University (awardee institution) and Profs. Wickramasinghe (co-PI, Chemical Engineering) and Qian (co-PI, Mechanical Engineering) at Colorado State University. In this research program, we are developing a new class of membranes that we call inverted colloidal crystal (ICC) membranes. They are highly ordered and periodic in structure, and easily functionalized. We are studying ICC membrane performance for (protein) separations and modeling how the membrane structure and surface chemistry impact separation performance. Our *long-term* research goal is to develop membranes for highly efficient separations with higher productivity than standard particle-based chromatography.

The rationale for this IREE supplement was to test run a new collaborative undergraduate program between Clemson University (CU) and Colorado State University (CSU). I-PRIME (Incubator Program for Research with International Mentors and Educators) will 'prime' undergraduates for international research. This program is designed to tie in with undergraduate research experiences promoted at CU and CSU. Students may be freshman, sophomores, or juniors. Freshman and sophomores participate for at least one year in existing research programs at CU or CSU to "PRIME" them for research abroad. The summer after their primer year of research, they conduct research abroad. Once established, the I-PRIME program could plug into other existing educational programs that have an international experience requirement.

Two undergraduate students and one early career assistant professor participated in this program. The students were Ruben Kemmerlin (CU) and Makenna Nielsen (CSU). The first point for student selection was that the students had to have completed 1 year of research prior to the start of the program. Ruben is a member of Prof. Husson's Advanced Membranes Research Group. Prior to selection, he had completed a research course, Engr 190, in which he learned about laboratory safety and techniques; the scientific method; the roles of observation, measurement, and experiment in science; research ethics; presentation skills and scientific communication; and team building. Ruben spent a minimum of 4 hours per week in the laboratory to work on his project. Makenna is a member of Prof. Wickramasinghe's research group and a member of the CSU Honors Program. Prior to selection, Makenna was enrolled in a research course conducting work under the mentorship of a postdoctoral researcher. A second point for consideration was an essay completed by students who wished to be considered for the IREE project. Using the essays and personal interactions as bases, Profs. Husson and Wickramasinghe selected these students.

The objectives of the IREE program were to 1) Prepare students for professional employment and graduate education in the global workplace, 2) Build strong international partnerships that enhance the research and education enterprises of all partners as a result of unique capabilities, 3) Test newly developed sulfonated pseudo-affinity membranes for purification of human influenza virus, 4) Extend the application potential of ICC membranes by modifying their surfaces with poly(ionic liquids) and measuring adsorption isotherms of carbon dioxide through the membranes, 5) Begin to investigate the conformational structures of modified membrane surfaces and their potential impact on binding and diffusion properties using molecular dynamics simulations. Objective 5 was specific to the work of the early career Assistant Professor, Prof. Qian. All of these objectives were complementary to the objectives of the main program.

Our host laboratory for the IREE project was that of Prof. Andreas Seidel-Morgenstern, Director and Head of the Physical and Chemical Foundations of Process Engineering Research Group at Max Planck Institute of Dynamics of Complex Technical Systems and Chair, Chemical Process Engineering at Otto-von-Guericke-Universität, Magdeburg, Germany. His group determines physical and chemical data and parameters that are related to the chemical engineering and bioengineering processes investigated at the MPI. They also investigate several separation and reaction processes. Bioseparations with membranes is a key research area in this topic. Our students carried out research at MPI from May-July, 2008. Prof. Qian was at MPI for 3 weeks during May-June, 2008.

RESEARCH ACTIVITIES AND ACCOMPLISHMENTS OF THE INTERNATIONAL COOPERATION

The abridged objectives of the current NSF award are to

1. Design, synthesize, surface modify, and characterize ICC membranes.
2. Characterize the separation performance of the membranes for protein solutions.
3. Use molecular modeling techniques to provide insight on how best to modify the ICC membranes.
4. Involve an undergraduate research team in Objectives 1-3 and incorporate the outcomes of the research program into pre-college outreach to underrepresented minorities.

The IREE supplement expanded the scope of the original award to include studies on membrane surface modification with poly(ionic liquids) for use in CO_2 separations, modeling of conformational structures of temperature responsive PNIPAM nanolayers,and testing of new pseudo-affinity membranes developed by our collaborators at the MPI for human influenza virus purification. Also, the award allowed us to test run our I-PRIME undergraduate international research program, which was not part of the original proposal.

Measurements of CO_2 Adsorption on Surface-modified Membranes

Goals for the student working on this IREE project were to

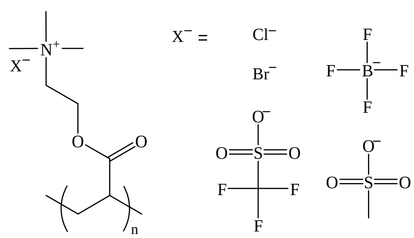
1. Use suface-initiated atom transfer radical polymerization (ATRP) to modify membranes with *polymerized* ionic liquid (pIL) nanolayers. Originally, we intended to use our ICC membranes; however, unexpected hurdles caused us to work with commercial macroporous membranes instead.
2. Measure adsorption isotherms of CO_2 in the pIL-modified membranes using the specially modified Wicke-Kallenbach cell and analysis methods developed by Prof. Seidel-Morgenstern's group at MPI.

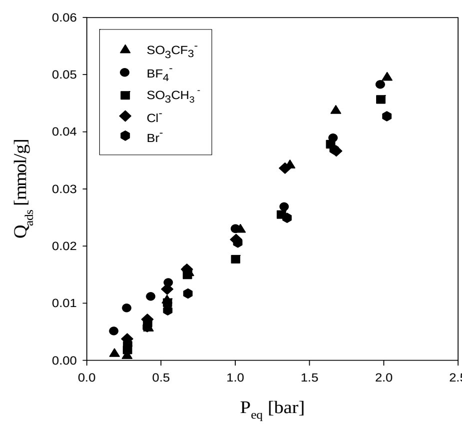
The rationale for the project was that pILs are outstanding candidates as membrane separation layers for selective CO_2 recovery from gas mixtures. To take advantage of pIL properties, we proposed that methods should be developed to graft polymerize uniform pIL nanolayers from membrane support surfaces.

The student accomplished both goals. Surface-initiated ATRP was used to modify cellulose UF membrane surfaces with poly([(methacryloyloxy)ethyl]trimethylammonium chloride). Polymerization time was a test variable. Thereafter, anion exchange was carried out to study the effect of pIL anion on CO_2 adsorption. Fig. 1 illustrates the pIL repeat unit and the five anions studied in this work. Adsorption isotherms were measured for CO_2 on modified and unmodified membranes. Adsorption temperature was an additional test variable. The results indicate that CO_2 adsorption increases as polymerization time increases and as adsorption temperature decreases. The impact of pIL anion was marginal. Control experiments with unmodified membranes showed no measurable adsorption of CO_2, indicating that CO_2 adsorption occurred specifically within the pIL nanolayer. A second control experiment showed no measureable adsorption of nitrogen as the test gas, indicating that the pIL nanolayers are selective for CO_2 adsorption.

Fig. 1. (below) pIL repeat unit and anions studied.

Fig. 2 (right) Impact of pIL anion on CO_2 adsorption isotherms at 25 °C.





To accomplish the work at MPI, the student was mentored by Prof. Seidel-Morgenstern. Laboratory training was done by a PhD student in Prof. Seidel-Morgenstern's lab, who also interacted with our IREE participant on a daily basis. During the IREE program, the student had unlimited access to the laboratory equipment that was needed to perform the measurements.

Purification of Human Influenza Virus

Originally, the second project was to use confocal laser scanning microscopy (CLSM) to visualize membrane pore structure and to visualize binding of fluorescently labeled proteins. However, the CLSM instrument was not operational when the project started, and a new project was developed. The main NSF project focuses on the development of membranes for bioseparations. At the MPI, a major focus is the development of cost effective purification processes for cell culture derived human influenza virus. It was decided then that *goals for the student working on this IREE project were to*

1. Validate analytical methods to determine recovery of human influenza virus after adsorptive membrane purification and to determine removal of contaminating host cell proteins.
2. Determine recovery of human influenza virus after adsorptive membrane purification.

The student spent the first half of her time in Germany learning how to use an ÅKTA Prime laboratory scale chromatography machine and validating viral assays. Using appropriate membrane holders, the ÅKTA was used to test membrane adsorbers. The student validated the hemagglutination Assay (HA), which is used to quantify the number of influenza virus particles. Influenza virus particles have surface or envelope proteins that are able to agglutinate human or chicken red blood cells. The aggregated RBCs form a visible lattice. It is an easy, simple and rapid method and can be applied to large amounts of samples. The student also learned how to run the single radial immunodiffusion (SRID) assay, which unlike the HA assay, determines the amount of antigenically active material present. Removal of contaminant host cell proteins was determined using the Bradford colorimetric protein assay.

The student ran a number of infective human influenza samples through sulfonated membranes. In general, recoveries were around 60-70%. Previous literature indicated that, unlike for non-enveloped virus particles (e.g. parvovirus), recovery of enveloped virus particles using adsorptive membranes is often problematic as elution of bound virus particles is difficult and incomplete [1]. The student's results do, however, indicate that significant removal of host cell proteins is possible. MPI researchers now have a better understanding of the performance of their new membranes and are working to improve them to obtain better recoveries.

Molecular Dynamics Simulation of Modified Membrane Surface

Goals for Prof. Qian were to begin simulation work to investigate the conformational structures of thermally responsive PNIPAM nanolayers.

The conformational structures of PNIPAM nanolayer with 50 degree of polymerization were investigated in water, 1 M NaCl, 1 M NaBr and 1 M HCl using classical molecular dynamics simulations. The simulation temperatures were chosen to be close to experimental low critical solution temperatures. The rationale for this work is that fundamental understanding gained from modeling and the insight obtained from structure- property relationship of the ICC membranes will allow us to optimize the performance of ICC membranes for specific separations

BROADER IMPACTS OF THE INTERNATIONAL COOPERATION

Research impacts

- The IREE supplement expanded the scope of the original award to include studies on membrane surface modification with poly(ionic liquids) for use in CO_2 separations, modeling of conformational structures of temperature responsive PNIPAM nanolayers, and testing of new pseudo-affinity membranes developed by our collaborators at the MPI. The MPI membranes were investigated for use as adsorptive membranes for purification of human influenza virus, an enveloped virus, which required the student to develop assays to determine the concentration of virus and host cell proteins. In addition, the award allowed us to test run our I-PRIME undergraduate international research program.
- By modifying membranes with CO_2-philic polymer and studying CO_2 adsorption, we gained information on how to advance the design of efficient CO_2 separation systems. S*ocietal impacts* of this phase of the work may include the reduction of greenhouse gas emissions, and the potential to use recovered CO_2 in enhanced oil recovery projects to increase oil production by existing reservoirs.
- We learned from modeling efforts that the conformational structures of PNIPAM depend strongly on the ionic strength, acidity, and the polarizbility of both the cations and anions in agreement with experimental findings. With this validation, we now can implement the modeling strategy to design modifier layers in a rationale way, rather than by adhoc rules of thumb.
- Our results indicate that adsorptive membranes could be used to purify human influenza virus produced via a cell culture method. Our results are significant for two important reasons: In the past human influenza virus particles for influenza vaccines, were produced using chicken eggs. Development of a more flexible cell culture based process is essential to meet peak demands for influenza vaccines. When developing new upstream production methods, it is essential to develop downstream purification processes concurrently in order to develop a robust manufacturing process. Our results are significant as we recovered intact influenza virus particle that could be used for vaccine development, and we used a cell culture based virus production process. Both of these results are of significant potential societal impact.
- The research was mutually beneficial: We had access to the MPI's world-class facilities for

bioseparations research and learned the latest techniques for measuring adsorption equilibria in porous media from Prof. Seidel-Morgenstern, who is a leading expert. Prof. Seidel-Morgenstern's group benefited from our expertise on membrane development and modification and computational modeling.

- The IREE supplement provided an opportunity to build on an existing collaboration between Prof. Wickramasinghe and MPI and to establish new collaborations between Prof. Husson and MPI and Prof. Qian and MPI. Prof. Qian is an assistant professor at CSU. She spent 3 weeks at the MPI conducting computational research and discussing ideas for collaboration with MPI researchers. Profs. Wickramasinghe and Husson spent 1 week in Germany as part of this program, during which they met with researchers at MPI to discuss opportunities for further collaboration. One outcome is a proposal that is being developed for submission to the NSF PIRE program, which includes the development of a collaborative degree between Otto-von-Guericke-Universität and CU and CSU.

Student impacts

- Two undergraduate students participated in this IREE program, including one female student. Neither student had prior experience working abroad. They had the opportunity to conduct research at one of the world-renowned Max Planck research institutes. The Max Planck Institute for Dynamics of Complex Technical Systems was established in Magdeburg in 1996; it is the first institute of the Max Planck Society dedicated to the engineering sciences and bridges the gap between basic research and industrial applications. Our students interacted with a highly internationally diverse population of about 170 employees and guest scientists who are working at the institute. The students have begun to understand the ways that engineering and science are conducted in another country. Such an educational experience will be invaluable for these students in their career.
- IREE students experienced issues such as language barriers and cultural differences. While researchers at the MPI are mainly English speaking, majority of the general populace of Magdeburg (formerly part of East Germany) is German speaking only. Students thus regularly had the opportunity to 'exercise' their German language skills.
- In addition to the research component of the IREE program, our students had the opportunity to visit many historically important buildings in Magdeburg, the 1200 year old capital city of Saxony-Anhalt, and to take day and weekend trips (by train) to various cultural attractions throughout Germany. Profs. Husson and Wickramasinghe arranged a trip to Quedlinburg, a medieval city of great cultural significance located in the Harz Mountains. Among the trips made by the students was one to Berlin, where they took a bus tour to get a guided, general overview tour of the city. They got their passports stamped at "Checkpoint Charlie" and took pictures of remains of the Berlin Wall.

DISCUSSION AND SUMMARY

The IREE supplement gave us the chance to test our I-PRIME program, which was a great success. The research visit to Germany was an enriching experience for both students academically, socially, and personally, and they are grateful to NSF and the host research group for the opportunity to have this learning experience. The I-PRIME program will be an integral part of our PIRE proposal to NSF.

As a result of this program, we know that surface-initiated ATRP can be used to modify cellulose ultrafiltration membrane surfaces with poly(ionic liquid) nanolayers, and that these membranes adsorb CO_2 selectively over N_2. Thus, our membranes can be used to separate CO_2 from gas mixtures. The results indicate that CO_2 adsorption increases as polymerization time increases and as adsorption temperature decreases. Five pIL anions were tested, and we learned that the impact of pIL anion was marginal.

We learned that the conformational structure of PNIPAM is very sensitive to the presence and concentrations of the ionic species in solution. The ionic strength and acidity have opposite effect on PNIPAM conformational structures.

From a scientific perspective we have shown that enveloped viruses may be purified using adsorptive membranes. In general, purification of enveloped viruses is difficult using chromatographic processes as attachment of the virus particles to the binding ligands can lead to damage to the lipid envelope or lead to incomplete elution of the bound virus particles. While our results do indicate low recoveries of bound viruses, we believe the recovery could be improved by careful design of the ligands.

The IREE program is an excellent mechanism for students and their advisors to build international collaborations. In our case, the fact that Profs. Husson and Wickramsinghe of the main NSF grant had existing collaborations with each other and that Prof. Wickramasinghe had initiated collaboration with Prof. Andreas Seidel-Morgenstern, ensured the successful outcomes from IREE funding. Thus, we submit that IREE supplements will have the biggest impact when the PIs have previous collaborations with the host institution. IREE funding will strengthen and extend these collaborations.

For students, we recommend attendance at a pre-trip workshop. In our case, both students attended the IREE pre-trip orientation offered by NSF, as well as university-sponsored study abroad orientation workshops. These workshops should occur well in advance of the research experience. For undergraduate students especially, we recommend that they conduct research at the home institution for 6-12 months prior to participation in IREE. Adjusting to cultural environment is difficult enough; this should not be compounded by limited experience in scientific research.

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BRIEF BIOGRAPHIES OF RESEARCHERS

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 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.

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 1997. He currently holds the positions of 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.

Xianghong Qian is currently an assistant professor in Mechanical Engineering at Colorado State University. She obtained her Ph.D. in theoretical chemistry in 1997 at the George Washington University. She did her postdoctoral research in theoretical condensed matter physics at the Max-Planck Institute for Microstructure Physics from 1997-2000. Her expertise includes first-principles calculations based on density-functional theory, *ab initio* and force-field based molecular dynamics simulations, metadynamics simulations, combined quantum mechanical and molecular mechanical (QM/MM) methods. Currently she leads an active research group at Colorado State University in computational materials science and engineering.

Ruben Kemmerlin is a junior chemical engineering student at Clemson University. He is a member of the Advanced Membranes Creative Inquiry research team advised by Prof. Husson. Mr. Kemmerlin was a Visiting Researcher at the Max Planck Institute for Dynamics of Complex Technical Systems in Magdeburg, Germany from May 2008-July 2008 as part of this IREE grant.

Makenna Nielsen is a junior chemical engineering student at Colorado State University. She is a member Prof. Wickramasinghe’s research team. Ms. Nielson was a Visiting Researcher at the Max Planck Institute for Dynamics of Complex Technical Systems in Magdeburg, Germany from May 2008-July 2008 as part of this IREE grant.