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Si Nanopores Development for External Control of Transport of Biomolecules

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Section A

1. Report on Training Progress

Nazar Ileri has been involved in an independent, multidisciplinary effort to create a new class of molecular sieves for proteins and viruses. Her experimental work has been performed concurrently at two campuses, LLNL and UC Davis, while theoretical components have been largely accomplished at UC Davis. As will be described, the devices she is creating have great potential to improve very significantly the efficiency and selectivity of molecular transport over what is presently available from state-of-the-art membranes.

Si-Based Ultra-uniform Nanoporous Filter Production and Surface Characterization

Over the course of the past year, Nazar Ileri has received training on membrane fabrication at LLNL under the supervision of Dr. Joe Tringe and Dr. Sonia Lėtant. In this time period, she has completed 24 mandatory safety training courses on lasers, chemicals and biological agents. At LLNL, she has been trained on spin coating of photoresist onto the substrate, pattern definition by interferometric lithography technique, metal deposition, together with some wet and dry etching processes. In addition, she learned to do surface characterization using atomic force micrsocpy (AFM) and scanning electron microscopy (SEM), which enabled her to obtain information about the uniformity of manufactured filters. Using these techniques, the trainee was successful in getting the first device with ~ 100nm diameter pore size. During the manufacturing processes, she has also been collaborating with Gratings Inc., the company that has performed the microfabrication of the membrane frame. Now, she is being trained on measuring the mechanical stability of fabricated membranes.

In Prof. Ahmet Palazoglu's group at UC Davis, the trainee has learned how to analyze images to acquire statistical data out of them. She has been trained on some image analysis programs such as Scion and Irfan View which enabled her to obtain quick data on size and uniformity of created patterns. She is now also being trained on Wavelet image analysis technique that has been proven successful for characterizing similarly sized periodic structures.

The training on production and surface characterization at both UC Davis and LLNL was successful in the first year, and Nazar presented her work at MRS meeting in San Francisco.

Transport Experiments

Prof. Pieter Stroeve's laboratory provided training on protein transport and separation experiments. The trainee performed preliminary transport experiments on the first devices and showed that small pyridine molecule transported through the pores with more than 15 times compared to the rate of commercial polycarbonate track-etched membranes with equivalent pore size. Using the same tools and techniques, she can investigate the separation of similar size molecules. Besides, she is now being trained to modify the surface charges to study surface charge and applied voltage effects on selectivity.

To train Nazar in non-technical skills, she has been working with two visiting graduate students from Ryukoku University in Japan and the National Taiwan University in Taiwan. Both students did collaborative research projects in Stroeve's laboratory. She trained Kohtaro Yamashita and Cieh Han Wu on diffusion experiments in membranes and on electroless gold coating of membranes.

Molecular Simulation and Computational Techniques

Nazar was trained on molecular modeling techniques in Prof. Roland Faller's group. She gained experience in using the Espresso package [1], and she can use the mixed Linux/Windows environment for her simulations. The trainee is performing her simulations using a DPD thermostat to ensure hydrodynamic accuracy and molecular dynamics over different time scales. With these tools, she developed her own techniques to create different pore geometries in filters and investigate the geometry effect on transport of biomolecules. At this point preliminary simulations are completed and the first production runs are starting. She is now also being trained on lattice Boltzmann simulations [2] and Coulombic interactions.

Nazar was awarded a Marie Curie grant to attend the Nanomemcourse “Nanostructured materials, membrane modelling and simulation”. She will also present a poster on her work in this workshop which will be held in June in Patras, Greece, where several distinguished members of the molecular modelling community will be present.

As a result of this training program, Nazar is becoming highly proficient in nanofabrication and chemical analysis methods, as well as in computational methods for understanding molecular transport mechanisms. Her preliminary results are highly encouraging, and her future devices are expected to demonstrate a new standard of performance for both efficiency and selectivity for nanoporous membranes.

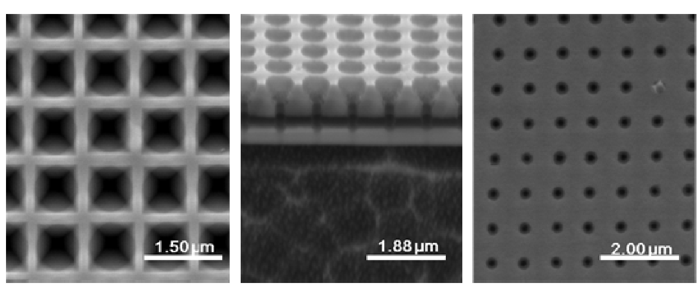
2. <u>Report on Research Progress</u>

Our biotechnology training program is based on an integrated study of the transport of biomolecules through conically-shaped, nanoporous silicon membranes. The overall objective of this effort is to demonstrate an efficient, highly selective membrane technology that is manufacturable for macroscopic areas and can be employed in sensing, diagnosic and biomedical applications. Our specific aims are to (1) fabricate and characterize the physical characterics of the membranes, (2) to demonstrate their utility for molecular transport and separation, and (3) to develop models that will facilitate understanding of these devices as well as improved performance of the next generation of devices.

We have proposed that the conical pores have superior performance characteristics compared to other porous filters. To study this hypothesis, complementary approaches from different disciplines, such as membrane synthesis, experiment, and molecular simulation need to be combined. This provides an ideal training environment for a future leader in biotechnology. Hence, for this study, Nazar Ileri has started to carry out a full range of experimental and theoretical investigations under our guidance. First, she has begun fabrication of filters with conical/pyramidal pores. She characterized the pores by AFM and SEM, and analyzed the images using wavelets and other mathetmatical tools. She has also started to conduct biomolecule transport experiments to compare the efficiency of fabricated filters vs. state-of-the-art commercial polycarbonate track-etched (PCTE) membranes. Finally, she has performed preliminary molecular calculations to investigate the operating principles of such systems and she has obtained results which she will present at the international “Nanostructured materials, membrane modelling and simulation” workshop in Greece.

Membrane Fabrication and Surface Characterization:

The trainee fabricated the first filters with ~100nm diameter conical pores using SOI wafers as substrates. To investigate the uniformity of the created structures, characterized the surface and analyzed the resulting images. Pore size variation decreased at least twice compared to those of PCTE with equivalent pore size. Nazar is doing further studies to improve the uniformity and decrease the pore size. She has optimized the laser exposure dose versus resist thickness and she is investigating metal deposition which plays a critical role on pore size and uniformity.



(a) (b) (c)

Figure 1. Scanning electron micrographs showing the top-down and cross-sectional views of the filter after (a) KOH etching to define the pyramidal Si pore shape, (b) and (c) HF etching to open the pore and enable molecular transport.

Biomolecule Transport Experiments

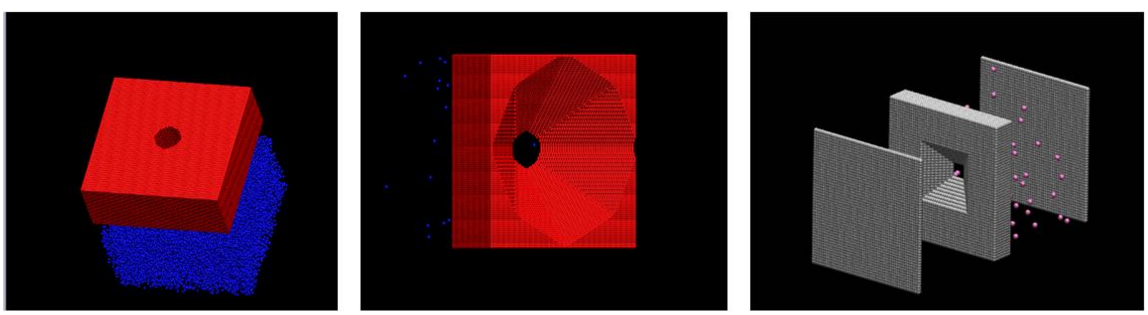
Nazar has investigated the efficiency of fabricated filters in comparison to PCTE membranes through a set of preliminary diffusion experiments. She used pyridine in the experiments and found that **this small molecule transported through the pores 15 times the rate of a comparable state-of-the-art PCTE membrane** with ~100 nm diameter pore size (see Table 1). This result, while preliminary, is nevertheless highly encouraging.

Table 1. Pyridine Flux through the membrane fabricated versus PCTE

	PCTE	**Our Membrane**
Pore size (nm)	**100**	**≤100**
Pore density (pores/cm^2)	**$6x10^8$**	**$3x10^8$**
Pyridine flux (mole/cm^{2}s)	**$5.5x10^{-10}$**	**$9.9x10^{-9}$**

Molecular Modeling

Nazar Ileri has focused on developing a mesoscale model to investigate the pore geometry effect on the diffusion of biomolecules. She has studied cylindrical, pyramidal and conical pores with large scale simulations and obtained transport parameters such as the mean square displacement, diffusion coefficients and density profiles of molecules.



(a) (b) (c)

Figure 2. (a) Cylindrical, (b) Conical (c) Pyramidal pores by simulation.

The tapered pores and thin membranes have potential for improving purification efficiency by more than a hundred-fold relative to existing methods, and will also reduce fouling. When successfully demonstrated, these new filters will dramatically improve the selectivity, speed and efficiency of many important processes, including protein ultrafiltration and purification, hazardous bio-molecule detection and drug discovery.

3. <u>Outcomes</u>

The trainee has learned a tremendous number of techniques in a short amount of time. She is becoming highly proficient in nanofabrication and chemical analysis methods, as well as in computational methods for understanding molecular transport mechanisms. Her experimental results were well-received when presented at the international Materials Research Society meeting in San Francisco in March. Her modeling efforts will be presented at the international "Nanostructured materials, membrane modelling and simulation" workshop in Greece in June.

Some preliminary data obtained under this training grant has been used to apply for a new grant from the LLNL Laboratory-Directed Research and Development (LDRD) program about the function of nanopore arrays in silicon. The proposal is under review at this time. We are currently applying for funding from the LLC Lab Fee Opportunity program to start a new research project evolving from the work of Nazar. Finally, we are also planning to submit proposals to (NIH) based on the work obtained here with the GREAT grant. SBIR grants will be pursued in collaboration with Gratings, Inc.

Nazar has been awarded the Marie Curie grant to attend the NanoMemCourse. In this training action of Marie Curie, she will not only present her preliminary work on molecular simulations on molecular transport in pores, but also she will have the opportunity to explore the potential for collaborative activities with other groups.

Future Plans:

We will fabricate and test a new generation of nanoporous membranes, demonstrating their superior molecular transport properties as well as their manufacturability. The design and fabrication of these devices will be guided by molecular dynamics simulations to determine the best pore shape and functionalization methods.

Collaborations formed as a result of this project and trainee

We have been in contact with Prof. Philippe Renaud at EPFL, Switzerland, to conduct some transport experiments there. Studies will be made for tracking a single molecule transport behavior through a single pore. For this purpose, a student from Prof. Renaud's lab will be exchanged with Nazar Ileri, and both students will apply for a International ICAM Junior Exchange Award.

Most significant impact:

This project has created prototypes of a powerful new molecular detection and separation device for security and biomedical applications.

Current information of GREAT trainee:

Nazar Ileri, PhD candidate CHMS,
Chemical Engineering and Material Science
B3086 Bainer Hall
One Shields Avenue
University of California Davis,
Davis, CA 95616.

4. Publications

Nazar presented her work at the Materials Research Society Spring Meeting in San Francisco, California: :

A Uniform Silicon Nanopore Array for Controlled Molecular Transport Nazar Ileri, Pieter Stroeve, Sonia Létant, Jerald Britten, Hoang Nguyen, Cindy Larson, Rodney Balhorn, Michael Shirk, Saleem Zaidi, Ahmet Palazoglu, Roland Faller and Joseph Tringe:, MRS Spring Meeting 2008 San Francisco, California.

She also presented posters at Biomembrane Frontiers in Davis 2008, the GREAT Fellowship annual retreat in Monterey 2007, and at the Joe Smith poster session, UC Davis, 2008, Davis, California.

5. Budget ??(will check with Carole to get the exact numbers)

Student

Student Salary
Benefits

Sponsor Allowance
Travel to GREAT retreat

Student Allowance
Supplies: Fluorescently labeled microbeads, wafer holders, tweezers, acetone air-brush for lift-off, epoxy, Kapton tape
Poster Prints
Travel to LLNL, GREAT Retreat, MRS, BPS Meeting

Detail: see below

OFFEROR The Regents of the University of California, Davis						
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR (PI/PD) Pieter Stroeve						
A. SENIOR PERSONNEL, PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in parentheses)	Man Hrs/Mo	Rates	- CAL	ACAD	SMR	Funds Requested Offeror
7. () TOTAL SENIOR PERSONNEL (1-6)						
B. OTHER PERSONNEL (SHOW NUMBERS IN PARENTHESES)						
1. () POST DOCTORAL ASSOCIATES						
3. (1) GRADUATE STUDENTS – Salary						
7. TOTAL SALARIES AND WAGES (A + B)						
C. FRINGE BENEFITS						
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)						
D. PERMANENT EQUIPMENT TOTAL PERMANENT EQUIPMENT						
E. TRAVEL (LIST ON BUDGET EXPLANATION PAGE)						
1. DOMESTIC (Great Retreat, BPS Meeting, MRS)						
2. FOREIGN						
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 2. TRAVEL 3. SUBSISTENCE Non resident tuition is not yet in this budget. It will be deducted at the end of the school year						

4. OTHER	
G. OTHER DIRECT COSTS (ITEMIZE ON BUDGET EXPLANATION PAGE)	
1. MATERIALS AND SUPPLIES 2 laptops, memory stick.Trehalose, DLPC, DSPC and cytochalamisn B.	
6. OTHER – Student Fees	

Section B

After getting the GREAT fellowship last year, a new stage in my career has begun . This wonderful opportunity has allowed me to initiate a multidisciplinary research experience that is I exactly wanted to do following my college education. It has been very rewarding for me to work both on simulations and experiments with experts from academia and industry, and to have the opportunity to learn about widely-varying fields of science. Over the past year, I worked in the laboratories of Prof. Pieter Stroeve, Prof. Roland Faller, and Prof. Ahmet Palazoglu at UC Davis, with Dr. Joe Tringe and Dr. Sonia Lètant at LLNL, and I have interacted frequently with Dr. Saleem Zaidi at Gratings, Inc..

At LLNL, I worked with Dr. Jerry Britten's group under the supervision of Dr. Tringe on interferometric lithography, performing critical fabrication steps of the membrane to create the pore arrays. Lasers were new to me, and I sincerely enjoyed my training there where I learned about these systems and then immediately applied my knew knowledge. Also at LLNL, I performed surface characterization experiments using atomic force microscopy (AFM) and scanning electron microscopy (SEM). In collaboration with Prof. Palazoglu, I then applied a series of image analysis techniques to get quantitative information about pore size and uniformity out of the SEM and AFM data.

At the UC Davis' Northern California Nanofabrication Center (NCNC), I learned how to perform additional important fabrication steps for the membrane, including as Cr and silicon oxide layer coatings using e-beam, and plasma-enhanced chemical vapor deposition (PECVD). These studies enabled me to optimize other parameters such as photoresist thickness, exposure dose, Cr layer deposition thickness, and reactive ion etch (RIE) etching times,,to get more uniform patterns and smaller pattern dimensions for the nanoporous membranes. In Dr. Lètant's lab at LLNL, I worked on KOH and HF etching, which are the final important steps in defining the size and geometry of the pore. Throughout this process, I collaborated closely with scientists at Gratings Inc., where the the frame of the membrane was made. Using the scaffold structure from Gratings, Inc., I was able to fabricate the first porous device with pore diameter ~100nm diameter.

In Prof. Stroeve's lab, I studied transport of biomolecules using the fabricated membrane together with state-of-the-art PCTE membranes, and and I compared the efficiencies of both filters. For these experiments, I used a UV-visible spectrophotometer and investigated the pure diffusion of small molecule pyridine through the pores. No surface modification was made on filters. In these preliminary experiments, I obtained 15 times higher diffusion rates with our membranes compared to commercial PCTE membrane having equivalent pore size. I have also started to do experiments to investigate the hindrance effect of cylindrical versus conical/pyramidal pores.

In Prof. Faller's lab, I worked on the production of different pore patterns, namely conical, cylindrical, pyramidal, and studied diffusion of molecules through these single pores. The simulations were run on a large time scale, applying the purely repulsive Weeks-Chandler-Andersen potential. Mean square displacements, flux, and diffusion coefficients were calculated for each case. These calculations allowed me to gain an understanding of operating principles for different shaped pores. Now we are in the process of determining the effect of columbic charges on transport of molecules.

Although all components of my studies are progressing well, each is now at different level of completion. More simulation work is necessary to support the experimental data, and the experimental devices must be be refined and scaled. Improved performance of the devices is anticipated as the modeling results inform the experimental results, and the experimental results in turn help calibrate and focus the models.

In the long term, my project aims are to improve the uniformity and decrease the pore size of fabricated devices, and to investigate the effect on molecular transport of parameters such as pH, applied voltage, and ionic strength. These investigations will be accomplished through a series of transport experiments as well as with simulations. We will prepare and submit papers at each successive stage of the work. Upon integration of all parts of the research next year, a much better understanding on the efficiency of this new class of filter will be achieved, and a new filtration technology will have been demonstrated. I believe this work will be highly benefical for for industrial and government applications, and at the same time will address important scientific questions about the fundamental nature of molecular transport through confined geometries. Finally, I plan to graduate by the end of my fourth year with these results and high-quality publications, and to then get a job either in academia or industry.

Supplies:

Fluorescently labeled microbeads, wafer holders, tweezers, acetone air-brush for lift-off, epoxy, Kapton tape. (?)

Expenses:

NCNC facility access charges, fed-ex charges for mailing samples to Gratings Inc., travel expenses to LLNL and MRS and BPS meetings, poster prints.

Computer programs learned:

ESPResSo, VMD

Conferences attended:

Joint Meeting of the Biophysical Society 52nd Annual Meeting & 16th International Biophysics Congress (IUPAB), February 2-6, 2008, Long Beach, California. In this meeting, I had a chance not only to follow the ongoing research projects on recent filter technology but also to exchange knowledge and to learn different approaches that I might also apply to my work. I found especially the work of Salvodor Mafe, Patricio Ramirez, and S.Z. Siwy on conical pores to be interesting and informative.

Materials Research Society Spring Meeting, March 24-28, 2008, San Francisco, California. I presented my work and attended many interesting talks such as Chaperonin Protein Scaffolds for Quantum dots by Xie, and Modification of Si surfaces by Nickel.

Presentations:

Nazar Ileri, Pieter Stroeve, Sonia Létant, Jerald Britten, Hoang Nguyen, Cindy Larson, Rodney Balhorn, Michael Shirk, Saleem Zaidi, Ahmet Palazoglu, Roland Faller and Joseph Tringe: A Uniform Silicon Nanopore Array for Controlled Molecular Transport, MRS Annual Meeting 2008 San Francisco, California.

Posters:

Nazar Ileri, Sonia Létant, Saleem Zaidi, Ahmet Palazoglu, Roland Faller, Joseph Tringe and Pieter Stroeve: External Control of Biomolecular Transport in Conical Si Nanopores, Biomembrane Frontiers, March 20, 2008, UC Davis, Davis, California.

Nazar Ileri, Pieter Stroeve, Joseph Tringe, Sonia Létant, Saleem Zaidi, Ahmet Palazoglu and Roland Faller: Si Nanopore Array Development for Externally-Controlled Biomolecular Transport, Joe Smith lecture, CHMS at UC Davis, February 29, 2008, Davis, California.

Nazar Ileri, Pieter Stroeve, Joseph Tringe, Sonia Létant, Saleem Zaidi, Ahmet Palazoglu and Roland Faller: Si Nanopore Array Development for Externally-Controlled Biomolecular Transport, Fourth Annual GREAT Retreat, October 28-29, 2007, Monterey, California.

Training Courses:

I will attend to the Nanomemcourse 'Nanostructured materials, membrane modelling and simulation' that will be held in Patras, Greece, in June, 2008. NanoMemCourse is a Marie Curie Conference and Training action, and one of its subcategories, the Nanostructured materials and membrane modeling and simulation, is held to deliver a set of lectures by the experts (Quirke, Weber, Rutledge etc.) on basic and advanced modeling and simulation methods for nanostructures. Further information on the workshop is available at http://www.nanomemcourse.eu/new/site/home/index.htm.

Interaction with Industry: We are collaborating with Gratings Inc. for the preparation of the frames for filters.

Granting efforts:
I have been awarded a grant to attend the NanoMemCourse in Greece in June 2008.

[1] H.-J. Limbach, A. Arnold, B. A. Mann, and C. Holm, "ESPResSo - An Extensible Simulation Package for Research on Soft Matter Systems," *Comput. Phys. Commun.*, vol. 174, pp. 704-729, 2006.

[2] P. Ahlrichs and B. Dünweg, "Simulation of a Single Polymer Chain in Solution by Combining Lattice Boltzmann and Molecular Dynamics," *J. Chem. Phys.*, vol. 111, pp. 8225-8239, 1999.

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