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Ravi Juluru
Munjal Patel
Muhammad Waqas Sadiq
Shabnam Sani
Chinmay Shukla*
Robert Stratford

*SPECIAL MESSAGE
The Microdialysis Focus Group shares the grief of the lives lost at the Sandy Hook School tragedy in Newtown, CT. With Probes magazine, we are spreading the light of knowledge.

*Correspondence should be addressed to Chinmay.Shukla@fda.hhs.gov
AAPS Microdialysis Focus Group
Steering Committee: Goals & Objectives

The Microdialysis steering committee consists of a Chair, Chair-Elect, Past-Chair and 11 steering committee members. The leadership begins with serving as a Chair-Elect, then moving on to the Chair and ends with serving as a Past-Chair, each of which is a 2 year commitment. All steering committee members serve for 2 years. The current MD FG steering committee including its leaders boasts a diverse group including 3 graduate students, 1 clinical scientist, 4 members from academia, 4 members from the industry and 2 members from the Government.

The goals and objectives of the Microdialysis Focus Group include:
- Designing programming for AAPS Annual Meeting and NBC conference
- Attracting more members to the MD FG
- Encouraging student participation
- Collaboration with other FGs for joint programming

The MD FG is realizing its goals and objectives by active discussions via a teleconference held once every 2 months, managing a MD LinkedIn group, attending the NBC and Annual Meeting Jamborees, and developing programs for AAPS meetings and AAPS co-sponsored meetings, etc.

Probes magazine: The Microdialysis Focus Group steering committee proudly presents the second issue of the Focus Group magazine, “Probes”. This magazine provides an update on the activities of the MD FG. Starting with this issue, the MD FG has decided to include the profile of a Doctoral candidate or a Post-doctoral scientist with the aim of realizing AAPS’ goal of active student and young scientist participation. Also included for your reading under our “Special Feature” section is a brief overview of the application of MD in ocular drug pharmacokinetics. Under our “Pearls of Wisdom” section, every issue will feature a profile of leading scientist in the field of MD to encourage and motivate young scientists. This issue proudly presents the profile of Dr. Isadore (Izzy) Kanfer, who is a recognized leader in addressing bioavailability and bioequivalency of dermal products using MD.
Microdialysis Technique Overview - Probe Types

By Shabnam Sani, Pharm D, PhD, Department of Pharmaceutical and Administrative Sciences, College of Pharmacy, Western New England University

Microdialysis probes are designed to mimic a blood capillary. Probes have a semi-permeable membrane that, when perfused with an isotonic solution which mimics the ionic composition of the surrounding medium of the probe, allows an exchange of compounds from an area of high concentration to an area of low concentration. A representative proportion of molecules from the extracellular fluid is then washed away by the constant flow of the perfusion fluid to the outlet of the probe to be collected and analyzed (1).

Microdialysis probes are generally commercially available in various sizes, designs and materials and consist of an inlet tube, a semi-permeable membrane, and an outlet tube. All dialysis membranes are made of highly biocompatible and inert materials, such as cellulose acetate, regenerated cellulose, polycarbonate, polyacrylonitrile, and polyether sulfonate copolymer (1, 3). There are basically four designs of probe geometries: loop, side-by-side, linear, and concentric (3) (Fig. 1).

The choice of probe is partly based on the site of implantation. For sampling of moving soft tissues such as muscle, heart, skin, liver, tumor, and adipose tissue, linear and loop styles are typically more suitable (2,3). The linear flexible design makes this probe more useful for implantation in peripheral tissue. Due to its flexibility, the linear probe easily

Fig. 1. Microdialysis probe designs: schematic illustration of four basic geometries. The dashed area represents the dialysis membrane. Arrows refer to the direction of flow of perfusate through probes.
enters into and exits from the tissue and allows positioning of the dialysis window over a specific site of interest (2). Concentric probes are very rigid in nature and best suited for intracerebral implantation because their structure provides the necessary mechanical stability and ruggedness for precise probe implantation which allows for in vivo sampling of specific brain regions in conscious, freely moving animals. This type of probe is very popular and most commonly used in the microdialysis literature for investigation in brain (3). Side-by-side probes are modifications of the concentric probe made of non-metallic flexible material and well suited for intravenous sampling. Probes have a wide range of molecular weight cutoffs from 5 to 100 kDa, thus allowing different sizes of biomolecules to pass through and be sampled (2).

Membrane-based sampling systems often encounter problems when sampling high molecular weight or highly lipophilic compounds in the interstitial fluid. Recently a novel “open flow microperfusion (OFM)” sampling system has been developed to overcome these problems by replacing the membrane with a steel mesh featuring macroscopic openings in combination with a peristaltic OFM pump in push/pull mode to achieve stable recovery of OFM samples (4). This system has been successfully used for clinical studies in dermis and to assess neuropeptides and large molecules in free-moving mouse (4-6). Currently, stable sampling and quantification of lipophilic and high molecular weight substances in the interstitial fluid can be performed in three tissues: adipose (aOFM, since 1995), dermal (dOFM, since 2006), and cerebral (cOFM, since 2008). To date, a linear OFM probe has been used in clinical studies in the dermis (dOFM) and adipose tissue (aOFM), while a concentric OFM probe in brain tissue (cOFM) is being used in preclinical studies (4). The OFM concept has the potential to be used for not only pharmaceutical research in a clinical in vivo setting but also in preclinical in vivo and ex vivo settings (4).

References:
Successful development of ocular drugs must address the challenges of drug delivery imposed by the eye’s unique anatomy and physiology. The clinical outcomes of a therapeutic strategy designed to treat anterior and posterior segment diseases can be determined by monitoring the drug concentrations in ocular fluids. Complete pharmacokinetic data on ocular disposition of drugs is scarce due to inaccessibility of aqueous and vitreous humor for continuous sampling. The technique of ocular microdialysis has gained popularity in the last two decades due to its ability to monitor drug concentrations in a continuous fashion with substantially fewer animals. Ocular microdialysis is an invasive technique and hence these studies are restricted to animal models. This special feature intends to highlight a few aspects of microdialysis and its relevance in studying the ocular disposition of drugs.

Ambiguity in the estimation of pharmacokinetic parameters often hinders the design of novel drug delivery systems to the eye. Ocular fluids are relatively inaccessible for continuous sampling of drugs [1]. By and large, ocular pharmacokinetic parameters are calculated by pooling data obtained from several subjects (6-20 animals/time point) at different time points. Substantial inter and intra-subject variability inherent to this approach can result in inconsistent and inaccurate results. At least 100 animals are needed to account for the variability and obtain a complete pharmacokinetic profile, which is expensive and time consuming. In contrast, microdialysis provides a complete pharmacokinetic profile in a single animal, thus reducing the total number of animals required.

Microdialysis is a sampling technique that involves insertion of a microdialysis probe that permits continuous monitoring of unbound analyte(s) concentrations in the tissue [2]. The microdialysis probe is connected to the inlet and outlet tubing as shown in Fig. 1. Ocular microdialysis is a recent technique that requires
skill and expertise in order to prevent tissue inflammation at the site of probe implantation and to preserve visual function [3]. Anatomy of the eye provides two sampling sites (aqueous and vitreous chambers) for microdialysis [4]. The ocular microdialysis technique was first reported by Gunnarson et al. [5] for measuring free amino acids in the pre-retinal vitreous space. Rabbits are most widely used because of the anatomical and physiological similarities to the human eye, and they are easy to handle and relatively inexpensive compared to other animals [3]. However, pharmacokinetic parameters obtained from rabbits may not be extrapolated directly to humans due to some differences, which include: lower corneal thickness, presence of nictitating membrane, slower blink reflex, and absence of a uveoscleral outflow pathway.

The microdialysis technique generates more precise pharmacokinetic data with fewer animals than conventional sampling [3]. Moreover, this technique does not require sample preparation/extraction, as the molecular weight cut-off of the probe excludes cellular protein from diffusing into the dialysate. Ocular pharmacokinetic studies using microdialysis are carried out in aqueous humor (anterior chamber microdialysis) or vitreous humor (posterior segment microdialysis). These techniques have been studied and validated in unconscious and conscious rabbit models [6-8].

**Anterior chamber microdialysis:** The majority of drugs are administered topically to the eye in the form of eye drops. Drugs absorbed through the cornea reach the aqueous humor, which serves as a sampling site. Drug concentration in aqueous humor can be correlated with concentrations in other anterior segment tissues [9, 10].

**Posterior segment microdialysis:** Vitreous humor acts as a sampling site for posterior segment diseases. Following intravitreal administration, drug molecules are rapidly cleared from the vitreous humor, depending on their molecular weight and polarity. Drug concentration in the vitreous humor can be correlated with the retina, and ocular microdialysis has been successfully utilized for vitreous sampling in rabbits [4, 11, 12].

Estimation of pharmacokinetic parameters based on the aqueous or vitreous humor concentrations alone may result in inconclusive results. For a complete understanding of drug disposition in the eye, a dual probe unconscious animal model capable of sampling both aqueous and vitreous chambers simultaneously was developed and validated by Macha and Mitra [6] (Fig. 2).

Pharmacokinetic parameters at the cellular or tissue level play an important role in understanding drug absorption, binding, and elimination, which in turn helps in determining dosages in the treatment of a particular disease. The ocular microdialysis technique involving the anterior and
posterior segments has provided a method for developing novel therapeutic strategies and examining their roles in enhancing ocular absorption. Many articles in the literature report the application of microdialysis as a tool for studying ocular drug disposition. However, most of these reports were published from a few research groups due to lack of expertise and limitations associated with the technique. Further refinement in the technique might encourage new investigators to employ ocular microdialysis for studying novel diagnostic and therapeutic agents, as well as optimizing drug development for the treatment of both anterior and posterior segment diseases.

References:

Dr. Isadore (Izzy) Kanfer was appointed to the position of Chair as the First Professor of Pharmaceutics at Rhodes University in 1980. He served as Head of Pharmacy at Rhodes University from 1987 – 1989 and as Dean of the Faculty from 1999-2007. Dr. Kanfer obtained his BSc (Pharmacy), BSc (Honours) and PhD in Pharmaceutics at Rhodes University and was Visiting Professor in Pharmaceutics at the University of California, San Francisco in 1980/81. In 1990 he spent a year as Visiting Professor in the Division of Pharmaceutics at the University of North Carolina’s School of Pharmacy in Chapel Hill.

Professor Kanfer spent several years in the Pharmaceutical Industry in Canada where he was Vice-President of Scientific Affairs at Genpharm Inc., Toronto. He also served as a representative of the International Generic Pharmaceutical Alliance (IGPA) for the World Health Organization’s (WHO) Committee on Multisource (Generic) Pharmaceutical Products and was a coauthor of that committee’s report to establish guidelines regarding registration requirements to establish interchangeability. He was appointed by the South African Minister of Health as a member of the South African Medicines Control Council (MCC) and served as a member of that Council from 1999 – 2004. He served as Chairperson of the MCC’s Expert Committee (Analytical) and chaired the MCC’s Complementary Medicines Committee. In addition, Professor Kanfer served as Vice-Chairperson of the MCC’s Pharmaceutical and Analytical Committee that establishes standards of bioavailability and bioequivalence.

Dr. Kanfer is a charter member of AAPS and is a founding member and Past-Chairman of the South African Academy of Pharmaceutical Sciences. He was an inaugural member of the editorial advisory board of the European Journal of Pharmaceutical Sciences and served as a member of the editorial board of the Journal of Pharmaceutical & Biomedical Analysis and is currently an Associate Editor of the Journal of Pharmacy & Pharmaceutical Sciences. Dr. Kanfer is also a member of the editorial board of the Encyclopedia of Pharmaceutical Sciences and Technology.
His research interests involve assessment of the quality, safety and efficacy of medicines, in particular their bioavailability and bioequivalence. More recently, he has focused on the development of methods for bioequivalence assessment of topical dermatological dosage forms wherein the drug is not intended to be absorbed into the systemic circulation.

Professor Kanfer established the Biopharmaceutics Research Institute (BRI) at Rhodes University and was its first Director. The BRI has successfully completed many bioequivalence studies on behalf of pharmaceutical companies that involve oral dosage forms as well as topical products containing corticosteroids, the latter using the human skin blanching assay (HSBA), also known as the Vasoconstrictor Assay (VCA), in accordance with the US FDA’s guidance.

Dr. Kanfer has supervised 34 postgraduate students (MSc & PhD) and post-doctoral fellows in Pharmaceutical Sciences. He has contributed to over 200 research publications and conference presentations and is co-editor of 4 books in the series, Generic Drug Product Development, in which the first book, Solid Oral Dosage Forms, was published in 2004, Bioequivalence Issues published in 2007, and more recently, International Regulatory Requirements for Bioequivalence and Specialty Dosage Forms published in January 2010. Professor Kanfer was the recipient of the Rhodes University Vice Chancellor’s Distinguished Senior Research Award for 2007. He has served as a consultant to various pharmaceutical companies and is an honorary life member of the South African Academy of Pharmaceutical Sciences and a Fellow of the South African Pharmaceutical Society. In 1998 he was elected to the Executive Committee of the Canadian Society of Pharmaceutical Sciences (CSPS) and has served as the Chairperson of the Heads of Pharmacy Schools Committee (South Africa) from 2000–2004. He was elected as a Fellow of the American Association of Pharmaceutical Scientists (AAPS) in 2010 and as Chair of the AAPS Bioequivalence Focus Group from 2010 – 2012. In 2008, he was appointed as Dean/Professor Emeritus (Pharmacy), Rhodes University, and in 2010 as Honorary Professor, KLE University, Belgaum, Karnataka, India.
Izzy Kanfer in conversation with Ravi Juluru

**Question:** Dr. Kanfer, Can you share with us your experience with microdialysis in your distinguished career at Rhodes University?

**Response:** Apart from topical corticosteroid products, in which the Human Skin Blanching Assay (HSBA), also known as the Vasoconstrictor Assay, can be used to assess the bioequivalence of topical corticosteroid products, assessment of bioequivalence of most other topical products (with the exception of transdermal/patch products in which the drug is intended to be absorbed into the systemic circulation) requires a clinical trial. Such a study generally involves monitoring a clinical end-point and comparing those clinical responses between a test (generic product) and reference product (RLD) (from the FDA’s Reference Listed Drug) and, in some instances, a placebo. However, clinical trials are not only expensive and time consuming, but clinical end-point results are often ambiguous and highly variable. Hence there is an urgent need to develop and validate novel methods which should be efficient and economic for use to determine the bioequivalence of topical products in general. At Rhodes University, since we have had many years of experience and collected a large amount of data from HSBA’s on a wide range of corticosteroid products, we decided to redo some of those bioequivalence studies using other methods such as dermal microdialysis (DMD) and tape stripping (TS). The objective was to see whether the data obtained from the HSBA on a specific corticosteroid product (Test & RLD) could be correlated with data obtained using DMD and/or TS. Our results using a TS method showed that TS has great promise and potential for use as an alternative approach for the bioequivalence assessment of topical clobetasol propionate formulations (Comparison of Tape Stripping with the Human Skin Blanching Assay for the Bioequivalence Assessment of Topical Clobetasol Propionate Formulations, Wai Ling Au, Michael Skinner, Isadore Kanfer. J Pharm Pharmaceut Sci (www.cspsCanada.org) 13(1):11-20, 2010). Unfortunately, using DMD, the limit of quantification (LOQ) for clobetasol propionate (CP) was not adequate to determine concentration in several samples, hence the bioequivalence of CP using DMD could not be determined. One important reason for the foregoing was that commercial CP products contain a very low concentration of CP (0.05%). Notwithstanding, in order to assess the viability and potential of DMD to monitor CP following application of CP to
the skin of human subjects, a subsequent DMD study was undertaken wherein a relatively high concentration of CP (4% ethanolic solution) was applied to the skin. In this case, the LOQ of CP was adequate to measure concentrations of CP in DMD samples and we were able to measure the bioavailability and also the flux of CP through the skin. The results of that study were subsequently published, viz: Application of dermal microdialysis for the determination of bioavailability of clobetasol propionate applied to the skin of human subjects. Wai Ling Au, Michael F. Skinner, Eva M. Benfeldt, Roger K. Verbeeck, Isadore Kanfer. Skin Pharmacol Physiol., 25:17–24, (2012).

**Question:** Dr. Kanfer, how do you see the future use of microdialysis in topical research?

**Response:** Specifically, dermal microdialysis (DMD) has great potential for use in topical drug research wherein a dynamic and real-time profile of the drug at the target site can be obtained following application of drug to the skin. Measurement of drug concentrations in interstitial fluids will provide valuable information on topical bioavailability, flux measurements and diffusion mechanisms. Ultimately, provided analytical methods with the necessary LOQ to measure very low concentrations of drug in dialysis samples can be developed, DMD will have an important role to play in the assessment of the bioequivalence of some topically applied products. Such a development will have a significant impact by circumventing the need to undertake clinical end-point studies in patients and will result in a significant reduction in time and costs to develop topical drug products.

**Question:** Dr. Kanfer, what are the current challenges with microdialysis that you are facing?

**Response:** There are currently several challenging issues associated with the application of microdialysis to study bioavailability, bioequivalence and pharmacokinetics of drugs applied to the skin. Since many topical drugs are highly lipophilic, the usual aqueous perfusion/dialysis fluids such as normal saline cannot be used because of drug-solubility constraints. Hence suitable alternative perfusion media need to be identified. In view of the very low concentrations of drug found in interstitial fluids following application to the skin, analytical methods with very high sensitivity and associated with adequate accuracy and precision will need to be developed and validated.
A further challenge is the duration of a DMD study, which is generally quite short (4–5 hours) and thus yields relatively too few samples to be able to result in a complete concentration-time profile and thus insufficient data may be acquired to obtain important information. Ideally, the microdialysis procedure should continue for considerably longer than the 4–5 hours and samples should be able to be collected for extended periods of time. A possible solution to this problem would be to use smaller, portable, pumps that would allow subjects to become ambulatory for periods during a prolonged DMD perfusion.

**Notes from Ravi Juluru:** The interview with Dr. Kanfer ended amicably and the steering committee thanked Dr. Kanfer for sharing with us his “Pearls of Wisdom”.
Role of Microdialysis in the Research of Young Scientists - A Doctoral Candidate’s Profile

Role of microdialysis in my PhD thesis research
Muhammad Waqas Sadiq

Thesis Advisor: Margareta Hammarlund-Udénæs, Ph.D.
Translational PKPD Group
Department of Pharmaceutical Biosciences
Uppsala University
Uppsala, Sweden

The aim of my thesis was to study different methodological and pharmacokinetic aspects of drug transport and interactions at the blood-brain barrier (BBB). To describe the extent of transport of drugs across the BBB, the partition coefficient of unbound drug ($K_{puu}$) is the most relevant descriptor. It is the ratio of unbound drug in brain interstitial fluid to unbound drug in blood. Microdialysis is the only method with which we can measure the pharmacologically active, unbound, drug directly in the tissue of interest in freely moving animals.

During my thesis work we directly measured the $K_{puu}$ for oxymorphone, diphenhydramine and morphine in rats by placing microdialysis probes into striatum and in the jugular vein for unbound blood concentration measurements. Recoveries for the different drugs were estimated by retrodialysis during each experiment, using deuterated calibrators for the drugs. Diphenhydramine and oxymorphone gave $K_{puu}$ values of 5 and 2, respectively, which revealed the presence of an active uptake transport process for these drugs at the BBB. The presence of this active uptake transport directs towards the possibility of designing new pharmaceuticals
with the property of preferentially being concentrated in the brain to treat CNS diseases.

By using microdialysis we were also able to study possible interaction between diphenhydramine and oxycodone for the uptake transport at the BBB and the relevance of *in vitro* experiments in this regard. The concentrations possible *in vivo* are much smaller than those possible to use *in vitro*. The interactions seen *in vitro* could therefore not be observed *in vivo*. Consequently, *in vivo* drug interactions at the BBB are less likely. Microdialysis produces a large number of samples during one experiment but the amount of each sample is minute, containing very low concentrations of the drugs studied. While studying the BBB pharmacokinetics of morphine at very low concentration this problem was addressed by using Accelerated Mass Spectrometry for analysis of the microdialysis samples.

Microdialysis has been a great sampling tool during the 4 years of my thesis research period for studying and understanding the brain distribution of different drugs. Two of the articles have been published [1, 2], while one is submitted to Journal of Pharmaceutical Sciences [3].

**References:**

3. Sadiq, M.W., et al., Oxymorphone active uptake at the blood-brain barrier and population modeling of its pharmacokinetic-pharmacodynamic relationship. (Submitted)
Microdialysis Focus Group  
AAPS 2012 Annual Meeting Minutes

Date: 10/16/2012  
Time: 12:00 PM  
Location: W190A, McCormick Place, Chicago, IL

Members present: Robert Stratford, Jaydeep Mehta, Munjal Patel, Sivaram Kiran Vaka, Aiqun Li, Sam Roiko and Chinmay Shukla  
Members absent with prior notification: Shabnam Sani  
Members absent: Hu Zhang, Ravi Juluru, Thomas Cremers

Micodialysis FG Membership Meeting Agenda:

Introduction: Chinmay Shukla, Chair of the Micodialysis Focus Group (MFG), welcomed all existing and new members with an introduction speech and greetings.

Presentation by Chair Re: Activities of the MFG: 2011-2012

1. MFG membership survey: A snapshot the microdialysis focus group member survey conducted from January 26, 2012 to February 08, 2012 was presented and discussed. Efforts of Robert Stratford and Aiqun Li were recognized in the design of the survey.

2. Member Groups Coordination Committee (MGCC) Review: FG activities report is submitted to MGCC every three years for review. In the meeting chair disclosed the letter dated on March 30, 2012 by MGCC to MFG. Committee found the MFG very active and successful, and recommended broadening the scope of programming.

3. AAPS program submissions: The table including the titles of the programs submitted, its status (accepted or not accepted) and the FGs involved in collaboration was displayed and discussed.

4. Programming at AAPS cosponsored events: MDFG programming at AAPS cosponsored events was shared. Two programs were accepted at the following meetings:
   a. PITTCON Conference and Expo (APQ Section cosponsored), March 17-21, 2013, Philadelphia, PA, USA
   b. Product Quality Research Institute workshop, March 11-13, 2013, Rockville, MD, USA
5. **New Programming being developed**: Chair discussed all the tentative titles which are being developed by of the MFG. The chair can be contacted for a list of these.

6. **Probes Magazine**: The first issue of Probes magazine was displayed and the reaction of the public to Probes release was shared. Chinmay and his steering committee members acknowledged and recognized the MFG members who contributed to the contents of the Probes magazine. In the meeting the discussion was kept open for innovative ideas for the next issue of Probes. New incoming steering committee member, Abhay Joshi (Ph.D. student at Long Island University), suggested including the list of posters to be presented by the MFG in upcoming AAPS annual meetings. Chair also showed the exact location of finding the link to the magazine on the AAPS Microdialysis Focus Group website.

7. **AAPS MFG LinkedIn group**: MFG has started the AAPS MFG LinkedIn group which involves the active contribution of all the existing and new members. Efforts and initiative by Siva Vaka were recognized.

8. **AAPS New Book Release**: MFG was happy to announce the released of a new book titled “Micodialysis in Drug development” as a part of AAPS Advances in the Pharmaceutical Sciences Series.

9. **Functioning of MFG**: The Chair discussed the functioning style of MFG steering committee. Specifically he concentrated on the use of a “rolling leadership and one voice going out” technique. Help and guidance by past chairs Elizabeth deLange and Grazia Stagni and AAPS staff Maria Nadeau was acknowledged. The Chair invited the MDFG members to submit programming ideas via the steering committee.

10. **Outgoing and Incoming Steering Committee members**: The Chair and all the steering committee members appreciated the service provided by the outgoing members: Jaydeep Mehta, Munjal Patel and Hu Zhang. New Members were also announced: Sai Boddu, Hiren Patel, Abhay Joshi, Anroop Nair, Frank Sinner and Nimita Dave were welcomed by the MD FG members.

**Meeting Close**: Chinmay Shukla again expressed his sincere thanks to all the past chairs, Robert Stratford (Chair-Elect), steering committee members and Maria Nadeau (AAPS) for their direct and indirect help and support to make the MFG a big success in the past year.
Microdialysis-Related Upcoming Meetings

PQRI Workshop on the Evaluation of New and Generic Topical Drug Products—Current Challenges in Bioequivalence, Quality, and Novel Assessment Technologies

March 11-13, 2013
U.S. Pharmacopeia Meeting Center
Rockville, MD 20852

Potential of Microdialysis in Evaluation of Topical Drug Products—An Opportunity for Bioequivalence Determination of Topical Products
Eva Benfeldt, M.D.
University of Denmark

Symposium Title - Bioanalytical Method Validation: Concepts, Expectations and Challenges in Small Molecule and Macromolecule

- March 18, 2013
- 2:00 PM – 5:30 PM
For additional information on the program and registration, please visit the following webpage: