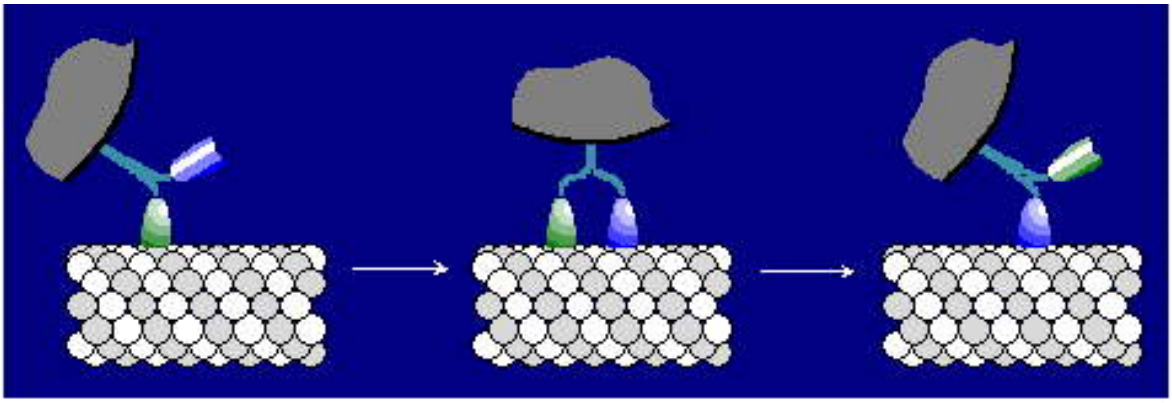
The 3rd Annual McGill Biophysical Chemistry Symposium

May 15, 2007 (Tuesday) 9:30-18:00
Otto Maass Chemistry Building
McGill University

Plenary Lecture
Jeff Gelles, Brandeis University



Invited Speakers:

David Burns (McGill University)
Christine DeWolf (Concordia University)
Yves De Koninck (Laval University)
Anne-Marie Lauzon (McGill University)
Stephen Michnick (University of Montreal)
John Pezacki (NRC)
Peter Swain (McGill University)

Program

9:30-10:30 **PLENARY LECTURE:** **Professor Jeff Gelles:** *"How kinesin keeps its grip: single-molecule studies of a molecular motor"* Department of Biochemistry, Brandeis University

10:30-10:50 **COFFEE BREAK, POSTER SETUP**

10:50-11:15 **Professor Yves DeKonick: "***Making sense of noise at live synapses in neurons***"** Neurophysics, Laval University

11:15-11:40 **Professor Christine DeWolf:** *"Raft formation by lipid second messengers: do phosphoinositide lipids phase separate in model membranes?"* Department of Chemistry and Biochemistry, Concordia University

11:35-12:05 **Professor Peter Swain:** *"Quantifying gene regulation in single cells"* Department of Departments of Physiology, Physics, & Mathematics, McGill University

12:05-14:00 **POSTER SESSION, PIZZA LUNCH**

14:00-14:25 **Professor Stephen Michnick:** *"Genomic-scale protein interaction network inference and dynamics"* Department of Biochemistry, Université de Montréal

14:25-14:50 **Professor Anne-Marie Lauzon:** *"Molecular mechanics of smooth muscle myosin in the latch-state"* Meakins-Christie Laboratories, Dept. of Medicine

14:50-15:10 **COFFEE BREAK AND POSTERS**

15:10-15:35 **Dr. John Pezacki:** *"Nanoscale imaging of beta-adrenergic receptors in the developing heart"* Steacie Institute of Molecular Sciences, National Research Council

15:35-16:00 **Professor David Burns: "***Biospectroscopy of Respiration: Applications from Womb to Tomb.***"** Department of Chemistry, McGill University

16:00-18:00 **POSTER SESSION AND CONCLUSION**

Supported by the Department of Chemistry, the Faculty of Science and the VP-research at McGill University, and by NeuroPhysics, University of Laval.

1. **Probing the Integrin-Actin Linkage using High Resolution Protein Velocity Mapping**
   David L. Kolin, Claire M. Brown, Jessica Zareno, Benedict Hebert, Leanna Whitmore, Alan Rick Horwitz and Paul W. Wiseman

2. **Design and characterization of new fluorescent probes for *in vivo* redox visualization**
   Paul Oleynik, Yoshihiro Ishihara, Amine Benmassaoud, Gonzalo Cosa

3. **Chiral Symmetry Breaking of Ethylenediamine Sulfate using Amino Acids**
   Jamie Surprenant, Ye Tao, Wendy Xu, Monica Cheung and Louis A. Cuccia

4. **Force spectroscopy study of chitosan**
   Marta Kocun, Dr. Louis Cuccia, Dr. Michel Grandbois

5. **INTERSTRAND CROSS-LINKED DNA**
   C. J. Wilds, E. Palus and A.M. Noronha

6. **Concerted dynamics link the DNA binding interface with the C terminal extension of the PBX homeodomain**
   Patrick Farber, Tara Sprules, Anthony Mittermaier

7. **Investigation of an intrinsic photonic amplification mechanism used for the ultrasensitive and sequence-specific detection of DNA material**
   Kim Doré, Hoang-Anh Ho, Denis Boudreau and Mario Leclerc

8. **Multifunctional Lipid/Quantum-Dots Hybrid Nanocontainers for Controlled Targeting of Live Cells**
   Gopakumar Gopalakrishnan, Christophe Danelon, Paulina Izewska, Michael Prummer, Pierre-Yves Bolinger, Isabelle Geissbühler, Davide Demurtas, Jacques Dubochet, and Horst Vogel

9. **Towards a Liposome Beacon:FRET Between Liposome Encapsulated Conjugated Polymers and Lipophilic Dyes**
   An Thien Ngo, Melanie Burger, Elisa Fuller, Pierre Karam, Gonzalo Cosa

10. **Single Molecule Fluorescence Studies on Liposome Encapsulated Light Emitting Polymers**
    Pierre Karam, Elisa Fuller, An Thien Ngo, Gonzalo Cosa

11. **Anchoring of vinculin to the membrane influences its binding strength in living cells**
    Gerold Diez, Phillip Kollmannsberger, Hojatollah Vali & Wolfgang H. Goldmann

12. **Improvement on Tethered Particle Motion**
    Hsiu-Fang Fan; Hung-Wen Li; Wayne Mah; Zhuo Li

13. **Fluorescence Moment Image Analysis of Ligand-induced Oligomerization of Growth Factor Receptors**
    Mikhail Sergeev, Santiago Costantino, Paul W. Wiseman

14. **Single-Molecule Studies of RecA kinetics on Duplex DNA**
    Axel Brilot, Wayne Mah, Hung-Wen Li

15. **Punching Holes in E. Coli**
    Mathieu Gauthier and Simon Rainville

16. **β-lactoglobulin and decyl-dimethyl-phosphine oxide (C_{10}DMPO) adsorption at the water/air interface.**
    Fei Xu, Dmitri O. Grigoriev, Sabine Siegmund, Reinhard Miller

17. **Investigations of Modified Gold Nanoparticle-Phospholipid Membrane Interactions to Screen for Biological Toxicity**
    Mohini Ramkaran, Antonella Badia

18. **pH-dependent insertion of Bt toxin into planar lipid bilayer**
    Nicolas Groulx, Marc Juteau, Jean-Louis Schwartz, Raynald Laprade and Rikard Blunck

19. **Dynamics of the 29.5 kDa Class A β-Lactamase PSE-4 by NMR**
    Sébastien Morin & Stéphane M Gagné

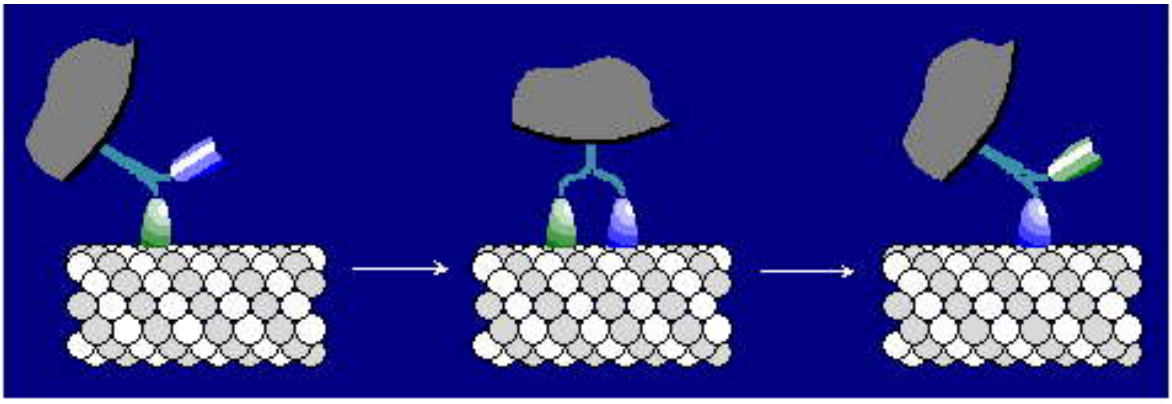
20. **Using FRET to quantify protein affinities in live cells: A comparison of methods**
    Catherine Lichten, May Simaan, Stephane Laporte, Peter Swain

21. **Characterisation of the structure and dynamics of truncated hemoglobin trHbN**
    Richard Daigle and Stéphane M. Gagné

22. **FCS-NSOM: A Unique Tool for Analysis of Single-Molecule Dynamics at High Concentrations**
    Dušan Vobornik, Zhengfang Lu, Daniel Banks, Cecile Fradin, Rod Taylor, Linda J. Johnston

23. **Structure and aggregation of recombinant silk proteins by solution nuclear magnetic resonance**
    Jérémie Leclerc, Isabelle Cloutier, Michel Pézolet, Stéphane M. Gagné, Michèle Auger

Abstract Oral Presentations



How kinesin keeps its grip: single-molecule studies of a molecular motor

Jeff Gelles
Faculty of Biochemistry, Brandeis University, P.O. Box 549110 415 South Street Waltham, MA 02454-9110

A single molecule of the enzyme kinesin is a chemically powered linear motor. Kinesin molecules catalyze a chemical reaction, the hydrolysis of adenosine triphosphate (ATP), and use a portion of the free energy released by this reaction to perform biologically useful mechanical work. Our work combines conventional biochemical methods with single-molecule biophysics approaches capable of observing nanometer-scale movements and chemical reaction events in individual kinesin molecules. These methods lead to new insights into the mechanism by which this remarkable molecular machine functions.

Making sense of noise at live synapses in neurons

Yves De Koninck
Centre de recherche Université Laval Robert-Giffard, Unité de neurobiologie cellulaire, 2601 Chemin de la Canardière, Suite F-6500, Beauport (Québec) G1J 2G3, Canada

Raft formation by lipid second messengers: do phosphoinositide lipids phase separate in model membranes?

C.E. DeWolf,
Department of Chemistry and Biochemistry, Concordia University

Phosphoinositides (PIs) are a minor group of membrane lipids that play a key role in signal transduction. It has been reported that these lipids are localized into rafts such that sufficient local concentrations are present for efficient enzymatic phosphorylation. It is unclear whether this raft formation results from the biophysical properties of the lipids themselves or if the lipids are sequestered into rafts through their interactions with proteins. We have used model systems to study the degree to which PIs form inositol-rich microdomains when mixed with other membrane lipids as a function of headgroup phosphorylation and chain saturation.

Quantifying gene regulation in single cells

Peter Swain
Department of Physiology, McGill University, 3655 Promenade Sir William Osler, Montreal, Quebec, Canada H3G 1Y6

Single cell measurements can be essential to determine network responses. For example, an all-or-nothing response may appear as graded at a population level. I will present in vivo measurements of the gene regulation function: the quantitative relation between transcription factor concentrations and the rate of protein production from downstream genes. By studying individual cells, we show that the gene regulation function fluctuates dynamically, thereby limiting the accuracy with which transcriptional genetic circuits can transfer signals. By following the asymmetric partitioning of fluorescence between daughter cells at each division, we are also able to estimate molecule numbers, and thus calibrate in vivo biochemical parameters in molecular units. Biochemical parameters and fluctuations, both their size and their lifetime, determine the effective single-cell gene regulation function.

Genomic-scale protein interaction network inference and dynamics

Stephen Michnick
Département de Biochimie, Université de Montreal, C.P. 6128, Succ. Centre-Ville
Montreal, Quebec, Canada H3C 3J7

Systematic studies of the organization of biochemical networks that make up the living cell can be defined by studying the organization and dynamics of protein-protein interaction. Here we describe recent conceptual and experimental advances that can achieve this aim and how chemical perturbations of interactions can be used to define the organization of biochemical networks. Resulting perturbation profiles and subcellular locations of interactions allow us to "place" each gene product at its relevant point in a network. We discuss how experimental strategies can be used in conjunction with other genome-wide analyses of physical and genetic protein interactions and gene transcription profiles to determine the dynamics of information flow through biochemical networks in the living cell. It is through such dynamic studies that the intricate networks that make up the chemical machinery of the cell will be revealed.

Molecular mechanics of smooth muscle myosin in the latch-state

Anne-Marie Lauzon
Meakins-Christie Laboratories, Dept. of Medicine, McGill University, 3626 St. Urbain Street Montreal, Quebec, H2X 2P2, Canada

Smooth muscle is found in all hollow organs of the body. It serves to maintain tension or propel content. As for any type of muscle, smooth muscle contraction is accomplished by the cyclic interaction between myosin, a molecular motor, and actin. This interaction is powered by the hydrolysis of MgATP.

Two isoforms of the smooth muscle myosin heavy chain are generated by alternative splicing of a single gene. They differ by the presence [(+)insert] or absence [(-)insert] of a 7 amino acid insert located in a surface loop above the MgATP binding pocket. Using an in vitro motility assay, we measured the velocity (ν_{max}) at which these two isoforms propel fluorescently labeled actin filaments. An approximately two-fold difference in ν_{max} was observed [(+)insert: 0.88±0.07 and (-)insert: 0.49±0.03μm/s]. To determine if this difference in ν_{max} was due to mechanical or kinetics differences between the two isoforms, we used the laser trap to measure the unitary displacement (*d*) generated by single myosin molecules and the attachment time (t_{on}) of myosin to actin. Briefly, a laser trap creates potential energy wells in which we can capture polystyrene beads in solution and to which we can biochemically attach an actin filament. The filament can then be micromanipulated and brought in contact with a single myosin molecule. The position of one of the beads can be measured using a photodiode quadrant detector. Mean-variance analysis of the resulting bead position vs time signals yields estimates of *d* and t_{on}. We found similar values of *d* for the two isoforms [9.6±1.0 for the (+)insert and 9.3±0.9 nm for the (-)insert] whereas an approximately two fold difference was seen for t_{on} between the (+)insert (158±19) and the (-)insert (301±32ms) isoforms, therby explaining the two fold difference in ν_{max}.

A unique property of smooth muscle is its ability to maintain force at low MgATP consumption. This property, called latch-state, is more prominent in slowly contracting tonic muscle [expressing mostly the (-)insert isoform] than in the rapidly contracting phasic smooth muscle [expressing mostly the (+)insert isoform]. Studies performed at the muscle strip level have suggested that tonic muscle myosin has a greater affinity for MgADP and therefore remains attached to actin longer allowing for cross-bridge deactivation (dephosphorylation) and latch-bridge formation. Another hypothesis states that dephosphorylated myosin reattaches to actin and maintains force. These properties of myosin have been inferred from measurements made at the tissue level but have never been verified at the molecular level. Therefore, we used the in vitro motility assay to measure ν_{max} when actin was propelled by (+) or (-)insert myosin, at increasing [MgADP]. A myosin mixture of 25% phosphorylated-75%unphosphorylated was used, to approximate in vivo steady-state conditions. The slope of ν_{max} vs [MgADP] was significantly greater for (-)insert myosin (-0.51±0.04) than (+)insert myosin (-0.15±0.04) demonstrating its greater affinity for MgADP. We then used a laser trap to measure the unbinding force from actin of unphosphorylated (+) and (-)insert myosin. Both myosin types attached to actin and their unbinding force (0.067pN ±0.013 for (+)insert myosin and 0.073pN ±0.018 for (-)insert myosin) was not significantly different. These results suggest that the greater affinity for MgADP of tonic muscle myosin and the reattachment of dephosphorylated myosin to actin can both contribute to the latch-state.

Nanoscale imaging of beta-adrenergic receptors in the developing heart

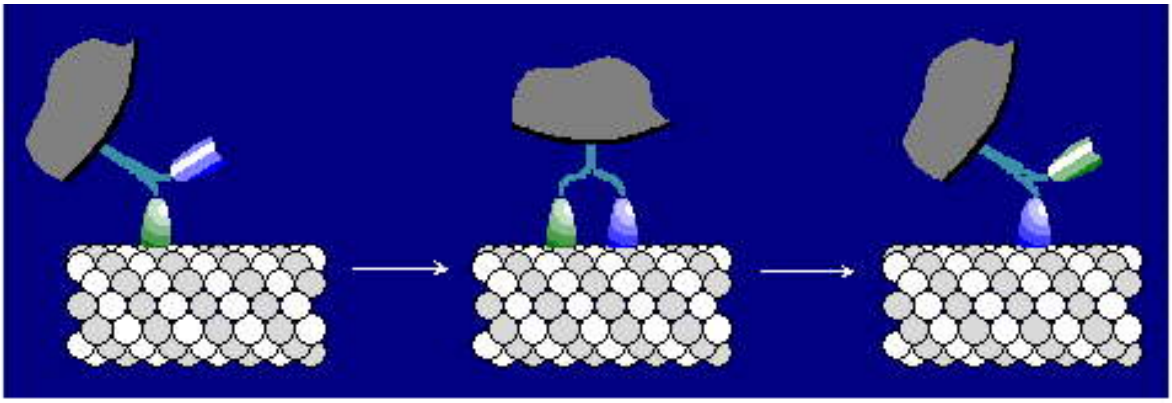
John Pezacki
The Steacie Institute for Molecular Sciences, National Research Council of Canada, 100 Sussex Drive, Ottawa, Ontario, Canada K1A OR6

In the mammalian heart the beating rate is controlled by an important signaling pathway that is initiated by the binding of signaling molecules to a membrane protein, the cardiac beta-adrenergic G-protein coupled receptor. Critical to these signaling events is the association of the adrenergic receptor with multi-protein complexes called signalosomes. The formation of the signalosome provides an element of spatial and temporal control over signaling that is required to regulate the beating of the heart. Using a combination of NSOM and fluorescence microscopy we have characterized the beta-adrenergic receptor signalosomes during a variety of conditions during murine heart development and embryonic stem cell differentiation. Using nano-scale imaging in combination with other model systems, including beta-adrenergic GFP(venus)-fusion proteins, we have quantitated the numbers and density of adrenergic receptors in cell membranes. The implications of these data on mammalian heart development and cardiovascular signaling will be discussed

Biospectroscopy of Respiration: Applications from Womb to Tomb

David Burns
Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montreal, QC, H3A 2K6, Canada

Abstract Poster Presentations



Probing the Integrin-Actin Linkage using High Resolution Protein Velocity Mapping

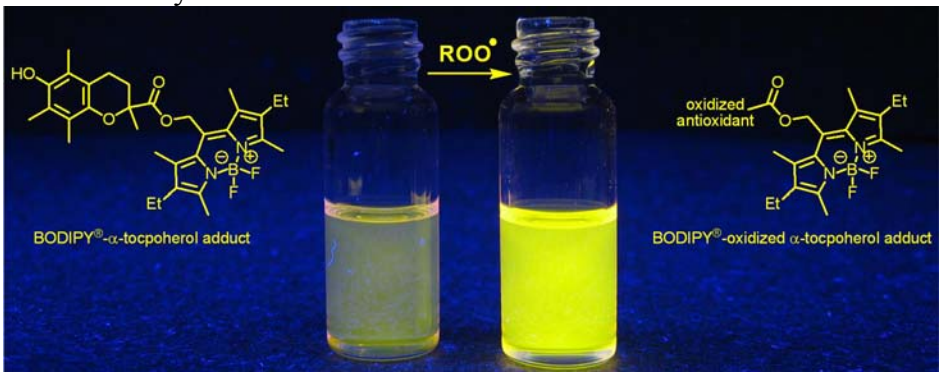
David L. Kolin[1], *Claire M. Brown*[1], *Jessica Zareno*[2], *Benedict Hebert*[1], *Leanna Whitmore*[2], *Alan Rick Horwitz*[2] *and Paul W. Wiseman*[1]
[1]McGill University, Montreal, QC, Canada, [2]University of Virginia, Charlottesville, VA, USA.

Cell migration is regulated in part by the connection between the substratum, integrins in the membrane and the actin cytoskeleton. Here we apply a novel image analysis tool, spatio-temporal image correlation spectroscopy, to quantify the directed movements of adhesion-related proteins and actin in membrane protrusions of migrating cells. We construct detailed cellular protein velocity maps for actin and the adhesion-related proteins α-actinin, α5-integrin, talin, paxillin, vinculin and focal adhesion kinase. From a comparison of velocities of the adhesion-related molecules to actin we characterize the efficiency of the linkage between integrin and actin in different cell types, and identify two likely points of slippage or disconnect in this linkage.

Design and characterization of new fluorescent probes for *in vivo* redox visualization

Paul Oleynik, Yoshihiro Ishihara, Amine Benmassaoud, Gonzalo Cosa
Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montreal, QC, H3A 2K6, Canada

Presented here are the design, synthesis, photophysical properties, and activity towards peroxyl radicals of a novel, non-invasive, α-tocopherol-mimicking, receptor-reporter type[7] fluorescent probe capable of spatial and temporal monitoring of the oxidative state of the system. The probe, titled B-TOH, consists of an α-tocopherol-mimicking receptor (Trolox®) tethered to a hydrophobic reporter flourophore (BODIPY 605®). The fluorescent probe initially has a low emission quantum yield, resulting from intramolecular quenching by the receptor moiety. Reaction of the receptor with peroxyl radicals leads to a *ca*. 9-fold increase in fluorescence of the reporter, thereby reporting, via emission, on the oxidative state of the system.



Chiral Symmetry Breaking of Ethylenediamine Sulfate using Amino Acids

Jamie Surprenant, Ye Tao, Wendy Xu, Monica Cheung and Louis A. Cuccia
Department of Chemistry & Biochemistry, Concordia University, 7141 Sherbrooke St. West, Montréal, Québec, CANADA, H4B 1R6

The chiral symmetry breaking process of ethylenediamine sulfate (EDS) was studied in the presence of various amino acids. EDS was stirred in its saturated solution with a chiral amino acid in the presence of grinding media. Preliminary results show a complete symmetry breaking of EDS crystals in the presence of an L-amino acid, whereas crystals of the opposite chirality are obtained in the presence of the D-amino acid. Further studies will be performed to determine the effect of other amino acids and sugars in the chiral symmetry breaking of EDS.

Force spectroscopy study of chitosan

Marta Kocun[*]*, Dr. Louis Cuccia*[*]*, Dr. Michel Grandbois*[‡]
[*]Department of Chemistry and Biochemistry, Concordia University, Montreal
[‡]Departement de Pharmacologie, Université de Sherbrooke, Sherbrooke

In the present study, force spectroscopy based on atomic force microscopy (AFM) is used to investigate adhesive properties of the polysaccharide chitosan. AFM was used to stretch and detach chitosan molecules from surfaces in order to study desorption and unfolding patterns. Well-defined features such as constant force plateaus and rupture peaks, were observed in the force curves. The lengths of the constant force plateaus obtained (50-200 nm) are consistent with the lengths of chitosan strands observed in high resolution AFM images. The desorption forces of single chitosan strands ranged from 40 to 300 pN depending upon the surfaces probed.

INTERSTRAND CROSS-LINKED DNA

C. J. Wilds, E. Palus and A.M. Noronha
Concordia University, Dept of Chemistry & Biochemistry,
7141 Sherbrooke St. W. MTL Qc H4B 1R6. cwilds@alcor.concordia.ca

DNA containing an interstrand cross-link (ICL) has been synthesized utilizing a bis-3'-*O*-phosphoramidite deoxythymidine dimer with the N^3 atoms bridged by a butyl linker. This methodology produces high yields of the desired ICL product, where the lesion can be engineered to be in different orientations. These duplexes will enable various biochemical and structural studies to elucidate ICL DNA repair. Thermal denaturation (T_m) studies of duplexes containing this cross-link in staggered 1-2 and 2-1 orientations show that these ICL duplexes are stabilized relative to non-crosslinked DNA while circular dichroism spectra and molecular modeling studies demonstrate little deviation from a B-form duplex.

Concerted dynamics link the DNA binding interface with the C terminal extension of the PBX homeodomain

Patrick Farber, Tara Sprules, Anthony Mittermaier
Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montreal, QC, H3A 2K6, Canada

Protein conformational changes can be an integral part of binding to substrates, however extracting quantitative data about these changes can be challenging. It has been previously shown that when the PBX homeodomain binds to DNA, it undergoes a disorder to order transition in which the C-terminus forms an α-helix. We performed backbone 15N CPMG relaxation dispersion experiments on the unbound PBX protein and found that there are concerted ms time-scale dynamics throughout the protein, including in the C-terminus, even in the absence of DNA. The data are well fit by a two-state model of exchange in which each site follows identical exchange kinetics. We propose that these dynamics are related to the conformational transition that occurs upon DNA binding.

Investigation of an intrinsic photonic amplification mechanism used for the ultrasensitive and sequence-specific detection of DNA material

Kim Doré1, Hoang-Anh Ho2, Denis Boudreau1 and Mario Leclerc2
1Département de chimie et Centre d'optique, photonique et laser (COPL), 2Département de chimie et Centre de Recherche en Sciences et Ingénierie des Macromolécules (CERSIM), Université Laval, Québec (QC), Canada, G1K 7P4

The interest for simple yet robust tools for the rapid detection of genetic or infectious diseases, via the transduction of hybridization of specific sequences of their genome, has grown tremendously in recent years. In particular, rapid detection is essential in order to accurately diagnose pathogenic diseases at the first stages of an infection. All biosensors developed up to now need either chemical modification of the analyte, complex instrumentation, or both. We present herein recent results on the development of a simple, ultrasensitive and sequence-specific DNA sensor based on simple electrostatic interactions between a cationic polymeric optical transducer and a fluorescently tagged negatively-charged nucleic acid target. The intrinsic photonic amplification mechanism is based on a fluorescence energy transfer between the polymeric transducer and the fluorophore tagged to the DNA probe which takes place in aggregates formed of several thousand polymer-DNA complexes. We will show how this amplification mechanism can easily detect as little as 20 double-strand DNA copies in 3 mL in the presence of the entire human genome in only 5 minutes, without the need for prior amplification of the target. Furthermore, we will show how the use of this sensor in pure water at 65°C allows to distinguish a single nucleotide polymorphism at similar concentration levels. Finally, we will present results from the investigation of this amplification mechanism by aggregate size determination and time-resolved fluorescence.

Multifunctional Lipid/Quantum-Dots Hybrid Nanocontainers for Controlled Targeting of Live Cells

Gopakumar Gopalakrishnan, Christophe Danelon, Paulina Izewska, Michael Prummer, Pierre-Yves Bolinger, Isabelle Geissbühler, Davide Demurtas, Jacques Dubochet, and Horst Vogel
EPF Lausanne, Switzerland

With tunable properties, quantum dot-lipid hybrid vesicles can be constructed for use as intracellular delivery vehicles as well as for the fluorescent staining of plasma membranes. Hydrophobic quantum dots have been incorporated into the bilayers membrane of lipid vesicles, offering a new way to image lipid vesicles, for selective delivery into either plasma membranes or the cytoplasm of living cells via appropriate membrane modification. The cell and lipid membranes can potentially integrate any kind of hydrophobic nanoparticle whose size matches the membrane thickness, thus opening possibilities for their manipulation in nanobiotechnology applications.

Towards a Liposome Beacon:
FRET Between Liposome Encapsulated Conjugated Polymers and Lipophilic Dyes

An Thien Ngo, Melanie Burger, Elisa Fuller, Pierre Karam, Gonzalo Cosa
Department of Chemistry, McGill University, Montréal, Québec H3A 2K6

Conjugated polymers (CP) exhibit highly sensitive fluorescence quenching that is ideal for detecting small amounts of analyte. Herein we report that liposome encapsulated CP may be used to detect interactions at the lipid bilayer surface via Förster Resonance Energy Transfer (FRET) between lipid-encapsulated CP and a lipid-intercalated dye. The fluorescence donor is the polymer sodium poly[2-(3 thienyl) ethyloxy-4-butylsulfonate] (PTEBS), and the acceptor is 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD). The dye is embedded in a dioleoylphosphatidylcholine (DOPC) bilayer surrounding the polymer molecule, where excess lipid is used to ensure encapsulation. Upon 440 nm excitation of the PTEBS-DOPC-DiD complex we readily observe DiD emission that is absent when the complexes are prepared without PTEBS. PTEBS encapsulation is verified by gel filtration and by quenching with methyl viologen. This system could be a precursor to studies of binding between ligands and membrane-embedded receptors.

Single Molecule Fluorescence Studies on Liposome Encapsulated Light Emitting Polymers

Pierre Karam, Elisa Fuller, An Thien Ngo, Gonzalo Cosa
Department of Chemistry, McGill University, Montréal, Québec H3A 2K6

New approaches are required to decipher the relevant reaction mechanisms in a lipid membrane because of its physical-chemical heterogeneity as a reaction medium. What is needed is a combination of a *"reaction" probe coupled to a reporting methodology* that allows one to determine precisely *where* the reaction occurs, *what* its elementary rate constant is, and *where* the reagents and products are. We have identified Single Molecule Spectroscopy as the ideal reporting methodology when combined with (*new*) *reactive fluorescent probes*.
Herein we will describe our new results obtained both at the ensemble and single molecule level, and involving the study of complex supramolecular structures consisting of lipid/light emitting polymers. These structures where conceived in an attempt to develop lipid-based biosensors. We will discuss our results within the context of the spectroscopic characterization of lipid/light emitting polymers, and their potential use as a biosensor platform.

Anchoring of vinculin to the membrane influences its binding strength in living cells

Gerold Diez1, Phillip Kollmannsberger1, Hojatollah Vali2 & Wolfgang H. Goldmann1
1) Center for Medical Physics and Technology, Biophysics Group, Friedrich-Alexander-University of Erlangen-Nuremberg, Germany
2)Department of Cell Biology and Dentistry, McGill University, Montreal, Canada

The focal adhesion (FA) protein vinculin (1066 residues) with its lipid-binding sites (residues 935-978, 1020-1040 and 1052 - 1066) plays an important role in cell migration and cell adhesion. Pull-down assays using artificial lipid membranes showed that the vinculin tail variant, lacking the lipid anchor (residues 1052 – 1066) is not able to interact with acidic phosphatidyl-serine (PS) or phosphatidyl-inositol (PI) vesicles under physiological conditions. *In vivo,* vinculin molecules, lacking this last 15 amino acides (vinUC) are still localized in the focal adhesion contacts, but form more stable FA in comparison to intact vinculin. In this work we investigated MEF vin(-/-) cells - transfected with EGFP linked vinUC – using the magnetic tweezer device. Our findings show that MEF vin(-/-) cells expressing vinUC are less stiff than MEF vin(-/-) rescue cells. During these measurements, more beads are disrupted from the integrin receptor in vinUC then in vinculin rescue cells, suggesting that the anchoring of vinculin to the lipid membrane is important for cellular binding strength.

Improvement on Tethered Particle Motion

Hsiu-Fang Fan; Hung-Wen Li; Wayne Mah; Zhuo Li
Department of Chemistry, McGill University, Montreal, Quebec, Canada.

Tethered Particle Motion (TPM) technique is a powerful method to study enzyme-DNA interactions at the single-molecule level. A surface tethered individual DNA molecule is attached at the distal end with a microscopic bead particle, and the bead undergoes Brownian motion. The amount of Brownian motion is limited by the DNA tether length. Earlier single-molecule experiments used either the standard deviation of successive bead centroid positions or the width of averaged bead image fitted with a Gaussian curve to correlate the DNA tether length. The precision of the TPM method thus relies on the uncertainty of Brownian motion. . We have developed a Force Tethered Particle Motion (FTPM) method to improve the resolution and extend the DNA range of tethered particle motion. In the FTPM experiment, a stretching force is applied to DNA-bead complex, and thus suppresses the bead's Brownian motion. The suppressed bead Brownian motion leads to smaller standard deviation of bead position measurement, and thus results in improved spatial resolution. Moreover the smaller Brownian motion under FTPM reduces the required number of frames required for the bead to explore all possible conformations; therefore it also leads to the improved temporal resolution. Finally, using equi-partition theorem in FTPM, the relation between variance and extension length can be predicted, thus no experimentally determined tether length calibration is required.

Fluorescence Moment Image Analysis of Ligand-induced Oligomerization of Growth Factor Receptors

Mikhail Sergeev, Santiago Costantino, Paul W. Wiseman
Departments of Chemistry and Physics, McGill University, Montreal, PQ, Canada

We present higher order moment analysis of fluorescence intensity fluctuations from individual laser scanning microscopy images applied to study monomer-oligomer distributions. We demonstrate that the number densities and brightness ratios of a mixed population of monomers and oligomers can be determined by analyzing higher order moments of the fluorescence intensity fluctuations from individual images for specific ranges of densities and particle brightness ratios. We implemented our technique to examine the distribution of aggregation states of PDGF-beta receptors on the surface of human foreskin fibroblast cells. The two-population moment analysis was applied to LSM images of cells incubated with human PDGF BB prior fixation. We could resolve two oligomerization states of the label on the cells: one for the specifically bound label and the other for non specifically bound label. The method was able to resolve a tetrameric population of the PDGF-beta receptors relative to the background distribution of non specifically bound fluorophore.

Single-Molecule Studies of RecA kinetics on Duplex DNA

Axel Brilot, Wayne Mah, Hung-Wen Li
Department of Chemistry, McGill University, Montreal, PQ, Canada

RecA is a key enzyme in the homologous recombinational repair pathway in E. coli. The objective of this study is to investigate the mechanisms of RecA mediated processes at the single molecule level, specifically RecA kinetics on double-stranded DNA (dsDNA) under different nucleotide states. We will use the tethered particle motion (TPM) technique to monitor the extension process. The TPM technique uses the Brownian motion of a tethered bead as an indicator of the length of the DNA strand tethering it to a surface. Data obtained to date show heterogeneous polymerization rates in the presence of the non-hydrolyzable ATP analogue ATPγS, as well as ATP hydrolysis dependent tether length fluctuations indicating polymerization and depolymerization, at low pH. In addition, the C-terminal deletion RecA mutant RecAΔC17 shows nucleation and extension at high pH, indicating a regulatory role of the C-terminal domain with respect to the dsDNA binding activity of RecA.

Punching Holes in E. Coli

Mathieu Gauthier and Simon Rainville
Department of Physics, Engineering Physics and Optics, Laval University, Pavillon Alexandre-Vachon, Québec, Québec, G1K 7P4

We are developing an in vitro system to study the bacterial flagellar motor. Tightly-focused femtosecond laser pulses are used to vaporize a submicrometer-sized hole in the wall of filamentous Escherichia coli. If the punctured cell is placed across a membrane dividing two volumes, one side will correspond to the exterior of the cell and the other side (where the bacterium is pierced) will correspond to the interior. We are working on experimentally realizing this by partly introducing a filamentous bacterium inside a micropipette and focusing our laser inside this pipette. We will thereby gain access to the inside of the cell and control over the proton-motive force. Encouraging preliminary results will be presented.

β-lactoglobulin and decyl-dimethyl-phosphine oxide (C_{10}DMPO) adsorption at the water/air interface.

Fei Xu[1], Dmitri O. Grigoriev[2], Sabine Siegmund[2], Reinhard Miller[2]
[1]Department of Chemistry and Biochemistry, Concordia University
[2]Department of Interfaces, Max Planck Institute of Colloids and Interfaces

Ellipsometry and surface tensionmetry were used to study adsorption behaviour of a bovine ß-lactoglobulin (BLG)/ decyl-dimethyl-phosphine oxide (C_{10}DMPO) mixture at the solution/air interface. Depending on the total concentration and mixing ratio the results suggest changes from thin to rather diffuse and thick mixed surface layers.

Investigations of Modified Gold Nanoparticle-Phospholipid Membrane Interactions to Screen for Biological Toxicity

Mohini Ramkaran, Antonella Badia
Department of Chemistry, Université de Montréal

Nanometer-scale materials have gained considerable attention for their potential applications in optoelectronics, storage devices, cosmetics/skin care formulations, as well as biomedical uses. As many of these applications are increasingly developed, there is great concern for the adverse health and environmental effects that these materials may impose. This work is therefore aimed at investigating the interactions of alkylthiolate-modified gold nanoparticles with biomimetic phospholipids monolayers and bilayers in order to address the possible connections between capping layer chemistry and potentially toxic interaction of the particles with cell membranes. In order to exemplify the different types of biological interactions (hydrophobic, hydrophilic, amphiphilic and electrostatic), various modified alkylthiolated gold nanoparticles have been synthesized and characterized. In addition, supported vesicular layers were formed to investigate nanoparticle-lipid interactions by scanning probe microscopy. The progress made thus far in this project will be presented.

pH-dependent insertion of Bt toxin into planar lipid bilayer

Nicolas Groulx[1,2], Marc Juteau[2], Jean-Louis Schwartz[2], Raynald Laprade[1,2] and Rikard Blunck[1,2]
[1]Department of Physics and [2]GÉPROM, Université de Montréal, Montréal, QC

Bt toxin (Cry1Aa) is a widely used biological pesticide, which forms cation-selective pores in bilayer membranes. The present study aims to investigate the structural determinants of its pore forming mechanism. It has been shown previously that the alpha-helix 4 plays a crucial role in pore formation. The charge-exchange mutation R131E at the N-terminus of helix 4 abolishes the pore formation in receptor containing vesicles at high pH, but allows for pore formation at neutral pH (*Vachon et al. AEM 2004*). Our preliminary results show that the pH dependence also holds true in planar lipid bilayers formed in the absence of receptors. While no pore formation was detected at pH 9, lowering the pH lead to rapid pore formation. Thus the pH-dependent step is not receptor binding but the subsequent translocation through the membrane.

Dynamics of the 29.5 kDa Class A β-Lactamase PSE-4 by NMR

Sébastien Morin & Stéphane M Gagné
Département de biochimie et de microbiologie, Faculté des sciences et de génie, Université Laval

The class A β-lactamase PSE-4 is involved in carbenicillin resistant infections. Understanding its mechanism presumes studying its dynamics. We therefore present a NMR backbone dynamics study of PSE-4. PSE-4 is an unusually ordered protein (ps-ns timescale) with most of this rigidity located around the active site. A network of μs-ms motions, which may be related to catalysis, is found throughout this enzyme exhibiting high stability for its two core domains. To complement data for the homologous TEM-1, we propose conformational rigidity as a general characteristic of class A β-lactamases.

Using FRET to quantify protein affinities in live cells: A comparison of methods

Catherine Lichten, May Simaan, Stephane Laporte, Peter Swain
Deaprtment of Physiology, McGill University, Div. of Experimental Medicine, McGill University

Fluorescence resonance energy transfer (FRET), the transfer of energy from one excited molecule to another, can be combined with fluorescence microscopy to visualize interactions between pairs of fluorescently tagged proteins. The extent of interaction between pairs of molecules can be estimated based on FRET signal strength but sources of error due to spectral overlap must be corrected for. We have developed a model of the photophysical events which take place during FRET imaging and used our model to assess the validity of formulas which are currently in use for analyzing FRET data.

Characterisation of the structure and dynamics of truncated hemoglobin trHbN

Richard Daigle and Stéphane M. Gagné
Department of Biochemistry and Microbiology, Laval University, Office 3251, Charles-Eugène-Marchand building, Québec (Québec), Canada, G1K7P4

The truncated hemoglobin trHbN protects the aerobic respiration of Mycobacterium tuberculosis by oxidizing nitric oxide into nitrate ion. The main objective of our research is to characterize the structure and the dynamic properties of trHbN. To achieve this goal we performed molecular dynamic simulations under various conditions. We present results obtained from simulations performed on the oxygenated and deoxygenated trHbN forms in which the conformational space of trHbN was explored.

FCS-NSOM: A Unique Tool for Analysis of Single-Molecule Dynamics at High Concentrations

Dušan Vobornik[1], Zhengfang Lu[1], Daniel Banks[2], Cecile Fradin[2], Rod Taylor[1], Linda J. Johnston[1]
[1]Steacie Institute for Molecular Sciences, National Research Council, Ottawa, ON K1A 0R6, Canada
[2]Department of Physics and Astronomy, McMaster University, Hamilton, ON L8S 4M1, Canada

Fluorescence Correlation Spectroscopy (FCS) is a technique that enables the study of the dynamics of single molecules. The prerequisite of FCS is to have, at any given time, only a few fluorescent molecules in the detection volume in order to generate significant fluorescence fluctuations due to single molecules dynamics. That is why common FCS setup based on confocal microscopy requires pico- to nanomolar concentrations of fluorophore. In biological systems concentrations are more often at a micromolar level.

Our Near-Field Scanning Optical Microscope (NSOM) uses an optical-fiber probe as a source of excitation light. One end of the fiber is tapered into a very sharp tip and the sample is illuminated through a small aperture at the end of the tip. The size of the aperture determines the size of the excitation volume. We can routinely make probes with an aperture diameter of around 50nm. That makes the NSOM excitation volume approximately an order of magnitude smaller than the confocal microscopes' volume.

Here we present the first demonstration of FCS-NSOM. Applications of the FCS-NSOM on biologically relevant systems are highlighted by measurements of lateral lipid diffusion in a model lipid membrane (mica supported DOPC lipid bilayers).

Structure and aggregation of recombinant silk proteins by solution nuclear magnetic resonance

Jérémie Leclerc[1], *Isabelle Cloutier*[1], *Michel Pézolet*[1], *Stéphane M. Gagné*[2], *Michèle Auger*[1]

[1]Département de chimie, CREFSIP, CERSIM, Université Laval, Québec, Canada G1K 7P4
[2]Département de biochimie et microbiologie, CREFSIP, Université Laval, Québec, Canada, G1K 7P4

Spider silk is a biomaterial with astonishing properties that compete with the best synthetic man made materials such as Kevlar. These mechanical properties confer to the spider silk several potential medical and military applications. The secondary structure that the two proteins adopt is known to be very important for the mechanical properties of silk. So our work is to study the structure of the proteins that make the spider web dragline by solution nuclear magnetic resonance (NMR) spectroscopy. The results show that the polyalanine segments adopt a structure with dihedral angles very close to β-sheet, the conformation adopted by the polyalanine in the silk fibers.