

Welcome!

In this, our third annual Chemical Biophysics Symposium, we hope to provide a relaxed forum for lively discussions and an opportunity to expand our horizons through interdisciplinary dialogue. The conference schedule tries to encourage the discussion of ongoing and controversial ideas in the field. The frequent coffee breaks allow informal discussion outside the bounds of a simple question-and-answer period, and the pub outings on Friday and Saturday nights offer further opportunities to interact with the other conference participants. In addition, by providing most meals we allow the attendees to concentrate on science. This culminates in our Saturday night banquet, which has a delicious menu and promises to provide some entertaining after-dinner distractions.

We are pleased to have researchers from many disciplines presenting their latest ideas and results at this conference. Furthermore, this meeting would certainly not be possible without the generous support of the Centre for Studies in Molecular Imaging, the Department of Chemistry, the Chemistry Club and Graduate Student Union, the Faculty of Arts and Science of the University of Toronto, and our many corporate sponsors.

We hope you enjoy your time at the conference. If you have any questions or concerns, please feel more than welcome to ask any member of the organizing committee – we are at your service. We look forward to a continuing tradition of exploration at the intersection of chemistry, biology and physics; the next symposium will be on **April 8-10, 2005**. See you next year!

The Organizing Committee

Cynthia Goh (Chair)	Darren Anderson (Student Chair)
Ray Kapral	Gabriel Hanna
Dwayne Miller	Jan Rainey
Jeremy Schofield	Bernie Sattin
Greg Scholes	Alexander Doust
David McMillen	Mark Sinyor
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	Jordan Dinglasan
	Nicolas Taulier
	Robin Stoodley
	Anja Nohe

CHEMICAL BIOPHYSICS SYMPOSIUM 2004

Department of Chemistry
University of Toronto
March 19-21, 2004

Friday, March 19

1:00–2:00pm Registration Koffler Centre Lobby

Session I Introduction: Cynthia Goh Koffler Auditorium
Sponsored by the Chemistry Colloquium Committee.

2:00–3:00 **Edward Yeung** (Iowa State University)
Imaging Single Molecules: Implications on Microenvironments and Disease Diagnosis

3:10–3:30 Coffee break

Session II Chair: Cecile Fradin Koffler Auditorium

3:30–4:15 a) **Terry Beveridge** (University of Guelph)
New Approaches to Study Bacterial Surfaces and Their Innate Ability to Interact with Environmental Metals and Nanominerals

4:15–4:35 b) **Kenneth H. Norwich** (University of Toronto)
The Saltiness of a Chloride Salt is Measured by its Molar Entropy

4:35–4:55 c) **David F. Green** (Massachusetts Institute of Technology)
Computational Design of a Protein Calcium Sensor for Single-cell MRI Imaging of Neural Activity

4:55–5:15 d) **Steve A. Mitchell** (Steacie Institute for Molecular Sciences, NRC)
Second Harmonic Generation Optical Activity of Interfacial Biomolecules

5:15–6:00 Panel/Audience Discussion: Topic: What insights into biology have been provided by computational and theoretical approaches?
Facilitated by TBA

Panelists: **Régis Pomès** (University of Toronto)
Fred Sachs (SUNY Buffalo)
Boris Steipe (University of Toronto)

6:00–7:30 Buffet dinner Davenport Atrium

Friday, March 19

<u>Session III</u>	Chair: Styliani Conostas	Koffler Auditorium
7:30–8:50pm	a) Christopher McKay (NASA-Ames) <i>Life in Extreme Environments and the Search for Life on Mars</i>	
8:50–9:10	b) Lisandro Hernandez de la Pena (Dalhousie University) <i>Quantum Effects in Liquid Water</i>	
9:15–??	<u>Informal Discussions</u> Live music by Erlenmeyer and the Flasks.	Faculty Club Pub

Saturday, March 20

<u>Session IV</u>	Chair: Gregory Scholes	Koffler Auditorium
9:00–9:45am	a) Robert Blankenship (Arizona State University) <i>Energy Collection by Photosynthetic Antenna Supercomplexes</i>	
9:45–10:05	b) Valentyn I. Prokhorenko (University of Toronto) <i>Photon-Echo Study of Electron-Phonon Coupling Strength in Molecules and Molecular Aggregates</i>	
10:05–10:25	c) Michael Knott (University of Toronto) <i>How Do Hydrogen Bonding and Hydrophobic Interactions Affect the Foldability and Cooperativity of Helical Proteins?</i>	
10:25–10:45	<u>Coffee break</u>	
<u>Session V</u>	Chair: Régis Pomès	Koffler Auditorium
10:45–11:30	a) Benoit Roux (Cornell University) <i>Absolute Free Energies: Theory and Computations</i>	
11:30–11:50	b) Bohdan J. Soltys (Amersham Biosciences Niagara Inc.) <i>Automated Microscopy and Image Analysis in Drug Discovery</i>	
11:50–12:10pm	c) Ulrich Hansmann (Michigan Technological University) <i>Generalized-Ensemble Simulations of Small Proteins</i>	
12:30–2:00	<u>Buffet lunch</u>	Davenport Atrium

Saturday, March 20

Session VI

Chair: David Cramb

Koffler Auditorium

2:00-2:45pm

a) **Stephen Ferguson** (University of Western Ontario)
Molecular Imaging Approaches to Cell Signalling

2:45-3:05

b) **Vali Raicu** (University of Toronto)
Studying Chemistry Inside the Living Cell: Proteins and their Interactions

3:05-3:25

c) **Shayamala Thinakaran** (University of Western Ontario)
Endocytosis of BMP Receptors is Mediated by Clathrin Coated Pits

3:25-3:45

d) **Catherine A. Greenhalgh** (University of Toronto)
Differentiation of Structures within Single Cardiomyocytes Based on Non-linear Multimodal Microscopy

Poster Session

Davenport Atrium

4:00-5:30

even-numbered posters

5:30-7:00

odd-numbered posters

7:00-??

Symposium Banquet

Restaurant Address ***

Do you know your stuff? Chemical Biophysics game show to follow!

Sunday, March 21

Session VII

Chair: Fred Sachs

Koffler Auditorium

9:30-10:15am

a) **David Weitz** (Harvard University)
Elasticity of Composite Actin Networks

10:15-10:55

b) **Mark E. Tuckerman** (New York University)
Enhanced Conformational Sampling via Novel Variable Transformations and Adiabatic Dynamics: Applications to Small Proteins and Drug Binding in the HIV-1 Protease

10:55-11:15

Coffee break

11:15-11:35

c) **Christa L. Brosseau** (Acadia University)
Analysis of the Absorption Behaviour of Mandelate Racemase and its Substrate: Comparison of Chromometry and EQCN Techniques

11:35-12:20pm

d) **Michelle D. Wang** (Cornell University)
Probing Gene Expression and Regulation at the Single Molecule Level

12:20-12:30

Closing Remarks

12:30

Lunch at a local restaurant (pay your own way)

Oral Presentation Abstracts

I **Imaging Single Molecules: Implications on Microenvironments and Disease Diagnosis**

Edward Yeung (*Department of Chemistry, Iowa State University*)

Due to intense research in the past few years there are now many approaches to single molecule detection. The next step is to design experiments to answer specific molecular questions that cannot be addressed by many-molecule experiments. Single fluorescent molecules in free solution can be followed directly as they diffuse and photodecompose. We are able to monitor single chromatographic events at a liquid/silica interface. This represents direct verification of the statistical theory of chromatographic retention. We will also describe a high-throughput imaging approach that allows determination of the individual electrophoretic mobilities of many molecules at a time. This opens up the possibility of screening DNA or proteins within single biological cells for disease markers without performing polymerase chain reaction.

IIa **New Approaches to Study Bacterial Surfaces and Their Innate Ability to Interact with Environmental Metals and Nanominerals**

Terry Beveridge (*Department of Microbiology, University of Guelph*)

Because bacteria are so small, it is extremely difficult to study them and their interactions with environmental materials at the single cell level. New approaches using cryo-transmission electron microscopy (cryoTEM) and atomic force microscopy (AFM) are providing a better understanding of the chemical and physical nature of bacterial surfaces. This information, when applied to the geochemistry of natural bacterial habitats, helps explain bacterial surface reactivity with soluble environmental metal ions, their adhesion to inanimate surfaces (including metal oxides), and their ability to formulate discrete nano-mineral phases.

IIb **The Saltiness of a Chloride Salt is Measured by its Molar Entropy**

Kenneth H. Norwich (*Professor, Physiology, Biomedical Engineering, and Physics, University of Toronto*)

We explore the hypothesis that the saltiness of an aqueous chloride solution is a linear function of the molar entropy of the salt in solution. When allowance is made for the absolute threshold of human taste for chlorides, this hypothesis leads to a simple equation governing the relative saltiness of any salt with respect to sodium chloride. The relative saltiness of any strong salt, say ACl to NaCl is defined as the natural logarithm of the ratio of the molarity of ACl to that of NaCl when the solutions taste equally salty to a human observer (saltiness matching). It is shown that, consistent with the known rules of perception, relative saltiness will be a linear function of the standard molar entropy of the salts ACl. Moreover, the slope of the emerging straight line will be equal to $1/(2R)$, where R is the gas constant, $8.3144 \text{ J} \cdot (\text{deg} \cdot \text{mole})^{-1}$. The theory can be validated with reference to measured data, and an approximate value for R can be calculated. Possibly this is the first time that a physical constant has emerged directly from a subjective measure made by the human senses. To dramatize the point, we can utilize psychophysical estimates of saltiness (less accurate than saltiness matching). Suppose, for example, that aqueous NaCl Solution 1 of molarity M_1 , tastes ‘twice as salty’ as Solution 2 of molarity M_2 . Then we have approximately $1/(2R) = (2/S_0) \ln(\sqrt{M_1}/M_2) + (1/S_0) \ln MW$ where S_0 is the standard molar entropy of sodium chloride and MW is the molarity of water. That is, the value of R is given approximately by a simple function of the molarities of the two tasted solutions and that of water.

IIc **Computational design of a protein calcium sensor for single-cell MRI imaging of neural activity**

David F. Green (*Massachusetts Institute of Technology*)

Computational protein design methods show great promise as a powerful tool for the development of novel reagents for the study of biological systems. Here, we describe the results of the application of some of these techniques to the design of a calcium-sensitive probe for use in MRI-based imaging of neural activity at the single-cell level. Taking the calmodulin–peptide binding system as an initial model, we design mutations to both binding partners so as to create a novel protein–peptide complex with minimal cross-reactivity to the wildtype complex. In this manner, we may develop a calcium sensing mechanism that, while based on calmodulin and sharing many properties with this evolved system, is orthogonal in its activity — the designed components will interact with one another, but not with the natural components. As a result, this reagent may be introduced into live organisms with minimal perturbations to the native cellular behavior.

IId **Second harmonic generation optical activity of interfacial biomolecules**

Steve A. Mitchell, Rich A. McAloney (*Steacie Institute for Molecular Sciences, NRC*)

Optical activity in second harmonic reflection from air/water interfaces with adsorbed biomolecules has been investigated. Results will be described for monolayer films of simple tryptophan (Trp) derivatives and peptides including alpha-helical polyamino acids. The second-order nonlinear susceptibility tensors have been fully characterized for simple Trp derivatives incorporating one and two Trp residues. Semi-empirical electronic structure calculations of molecular hyperpolarizabilities have been used to study the origin of the nonlinear response. Our results indicate that the chiral response is enhanced by charge-transfer interactions between indole and amide chromophores. Intramolecular coupling between Trp residues appears not to be important for the derivatives with two Trp residues. For alpha-helical polyamino acids, the nonlinear chiral response for the fundamental wavelength ~ 400 nm is associated with amide chromophores on the peptide backbone. Prospects for useful applications of second harmonic generation optical activity (SHG-OA) will be briefly considered.

IIIa **Life in Extreme Environments and the Search for Life on Mars**

Christopher McKay (*NASA-Ames*)

The most interesting astrobiological questions related to Mars deal with the nature of its early environment and its potential for the origin and development of life. Studies of Earth's early environment provide a basis for considering how life might have developed on Mars and where on Mars records of that early life may be found. Such records would include fossil evidence of life but fossils alone will not address questions as to the nature of life on Mars compared to Earth life. Life on Mars could have shared a common origin with life on Earth or it could represent a second genesis. The question of a second genesis of life on Mars can be resolved only if we can access biological materials from past or present organisms. There are places on Mars where such ancient biological material might be preserved.

IIIb **Quantum Effects in Liquid Water**

Lisandro Hernandez de la Pena, P. G. Kusalik (*Department of Chemistry, Dalhousie University*)

Water as a solvent is an essential element of many chemical and biochemical processes. Its peculiar properties have been extensively studied with a variety of experimental and theoretical tools, and therefore, well documented evidence exists on the significant difference between the equilibrium and dynamical properties of H₂O and D₂O. This difference is believed to be predominantly due to the change in the inertia moment of the molecule. Moreover, the thermal wavelength of the hydrogen atom can be expected to be non-negligible even at room temperature. Thus, there have been several computer simulation studies that have explored the role of the expected quantum effects. In this work, we present an extension of the centroid path integral molecular dynamics technique to the treatment of rigid bodies, and introduce definitions for rotational centroid and rotational uncertainty. We apply this technique to liquid water and report a number of equilibrium and dynamical properties of H₂O and D₂O. We discuss the importance of the quantization of the rotational motion by comparison with classical simulations and identify explicitly the mechanism for rotational tunneling in water. The similarity of our results in comparison with quantum simulations carried out on flexible models suggests that flexibility is of a minor importance in the description of the bulk properties of liquid water in a quantum simulation.

IVa **Energy Collection by Photosynthetic Antenna Supercomplexes**

Robert E. Blankenship (*Department of Chemistry and Biochemistry, Arizona State University*)

Photosynthetic antenna complexes function to absorb light energy and transfer excited state energy to reaction center complexes where electron transfer processes store the energy in the form of chemical bonds. Photosynthetic antennas are remarkably diverse in structure and pigment composition and certainly reflect multiple independent evolutionary inventions to solve the problem of how to collect and store the sun's energy. We utilize a wide range of techniques to study these systems, including ultrafast spectroscopy, microscopy, crystallography and biochemical analysis. This talk will include a general introduction to antenna complexes and then detailed descriptions of structure and excited state dynamics in two types of antenna systems, the photosystem 1 supercomplex from iron-stressed cyanobacteria and the chlorosome antenna from green photosynthetic bacteria. These represent two very different solutions to the energy capture problem.

IVb **Photon-Echo Study of Electron-Phonon Coupling Strength in Molecules and Molecular Aggregates**

Valentyn I. Prokhorenko (#,*), Rienk van Grondelle (*) , R.J. Dwayne Miller (#)
((#) *Departments of Chemistry and Physics, University of Toronto, Canada; (*)
Department of Biophysics, Faculty of Science, Vrije Universiteit Amsterdam, De
Boelelaan 1081, 1081 HV Amsterdam, The Netherlands*)

A method for direct measurement of the electron-phonon (system-bath) coupling strength in molecules and molecular assemblies is proposed and experimentally realized. The method is based on the three pulse photon echo technique with non-trivial time ordering of interacting laser pulses. It allows measuring the electron-phonon coupling strength, characterized by the Huang-Rhys Factor (HRF) even at room temperature. We applied this method (homodyne photon-echo detection) for the natural molecular aggregates (the chlorosomes, isolated from *Cf. aurantiacus* green bacteria) and found a surprisingly low magnitude of the HRF (ca. 1) at room temperature, as compare to those for the bacteriochlorophyll monomers in a solvent (HRF > 2.5). The possible physical origin of such low HRF, and the energy transfer in chlorosomes are discussed within recently developed advanced exciton theory. The possibility to enhance a sensitivity of proposed method using a heterodyne detection will be also discussed.

IVc **How do hydrogen bonding and hydrophobic interactions affect the foldability and cooperativity of helical proteins?**

Michael Knott, Hue Sun Chan (*University of Toronto*)

We present the results of Langevin dynamics simulations of a simplified atomic model of polypeptide chains, designed to form one-, two- and three-helix native conformations. The effect of the relative strengths of the hydrophobic and hydrogen bonding interactions is investigated. Provided that the two interactions are appropriately balanced, a simple potential function allows the chains to fold to their target structures. If the hydrophobic interaction is too strong, helix formation is preempted by hydrophobic collapse into molten-globule-like conformations.

The process of folding to the helical conformations exhibits certain cooperative features; the degree of cooperativity increases with polypeptide length, but falls far short of that observed experimentally for small 'two-state' proteins. Although the model produces a heat capacity peak associated with thermal unfolding, the energy distribution is not bimodal.

Our findings suggest that models with simple pairwise additive interaction schemes, involving hydrophobic interactions and hydrogen bonding, can mimic the folding of small helical proteins, but that such models are insufficient to produce high degrees of cooperativity. We discuss our results in the context of a recently proposed scenario for downhill folding.

Va **Absolute Free Energies: Theory and Computations**

Benoit Roux (*Department of Biochemistry, Weill Medical College, Cornell University*)

By and large, biological processes involving ligand binding specificity, protein-protein association, protein-membrane association, protonation and unprotonation of ionizable groups, and macromolecular conformational changes, are driven thermodynamically by a reversible work function, the free energy, or more generally by its configuration-dependent analog, the potential of mean force. To understand the behavior of biomolecules, one must be able to account accurately for the environment-mediated solvation free energies arising in diverse and complex systems (e.g., bulk aqueous solutions, the active site of an enzyme, or a bilayer membrane). A quantitative determination of free energies is, therefore, a problem of central importance in theoretical biophysics. Computational approaches at different level of complexity and sophistication can be used to try and address this problem. Those range from (relatively expensive) molecular dynamics free energy simulations (MD/FES) based on all-atom model in which the solvent is treated explicitly to (relatively inexpensive) Poisson-Boltzmann (PB) continuum electrostatic models in which the influence of the solvent is incorporated implicitly. Depending on the context, these theoretical approaches can all play a useful role trying to better understand biomolecular systems. We will describe recent advances in the calculation of solvation free energies and binding free energies for biomolecular systems.

Vb **Automated Microscopy and Image Analysis in Drug Discovery**

Bohdan J. Soltys, Yuriy Alexandrov, Louis Dagenais, Denis Remezov, Ahmad Yekta. (*Amersham Biosciences Niagara Inc.*)

Pharmaceutical screening of compound libraries is increasingly adopting novel sub-cellular imaging and fluorescent technologies to screen for drugs with specific molecular effects directly within living cells. These technologies enable fully automated cell screening in a robotic environment. At the core of the methods are sophisticated algorithms which extract information from extremely complex images, enabling characterization and quantification of molecular and cell population activity. We describe validated biological assays and algorithms developed on a robust screening system, the IN Cell Analyzer 1000. Recent work developing intelligent learning algorithms which mimic human thinking is also described. These learning algorithms are capable of categorizing cells and subcellular organelles into discrete classes or states. In comparisons with human scoring of the same images, the learning algorithms can correctly classify >90% of cells. Along with advances in image processing techniques, the implementation of machine intelligence to interpret complex images represents a necessary next advance in drug screening.

Vc **Generalized-Ensemble Simulations of Small Proteins**

Ulrich H.E. Hansmann (*Department of Physics, Michigan Technological University*)

The successful deciphering of the human genome has highlighted anew an old challenge in protein science: for most of the resolved protein sequences we do not know the corresponding structures and functions. Neither do we understand in detail the mechanism by which a protein folds into its biologically active form. Computer experiments offer one way to evaluate the sequence-structure relationship and the folding process but are extremely difficult for detailed protein models. This is because the energy landscape of all-atom protein models is characterized by a multitude of local minima separated by high energy barriers. Only over the last few years have been algorithms developed that allow one to overcome this multiple-minima problem in protein simulations. Prominent examples of these new and sophisticated techniques are parallel tempering and generalized-ensemble sampling. I will present applications of these methods to the simulation of small proteins such as the 34-residue PTH(1-34) and the 36-residue villin headpiece subdomain HP-36. At the end of my talk, I will discuss our current attempts to extend the size of proteins that can be studied in computer simulations.

References:

- 1) N.A. Alves and U.H.E. Hansmann, Phys. Rev. Lett., 84 (2000) 1836.
- 2) U.H.E. Hansmann and J.N. Onuchic, J. Chem. Phys. 115 (2001) 1601.
- 3) U.H.E. Hansmann and L. Wille, Phys. Rev. Lett., 88 (2002) 068105.
- 4) U.H.E. Hansmann, Comp. Sci. Eng., 5 (2003) 64.
- 5) C.-Y. Lin, C.-K. Hu and U.H.E. Hansmann, Proteins: Structure, Function and Genetics, 52 (2003) 436.

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VIa **Molecular Imaging Approaches to Cell Signalling**

Stephen Ferguson (*Department of Physiology and Pharmacology, University of Western Ontario*)

My research group is focused on unraveling the basic molecular mechanisms underlying the regulation of G protein-coupled receptor (GPCR) activity. To achieve this goal, we have combined biochemical, imaging and yeast two-hybrid genetic approaches to examine differences between the mechanisms regulating the, endocytosis, intracellular trafficking and signaling of four distinct GPCR subtypes: β 2-adrenergic receptors, angiotensin II type 1A receptors, fMLP receptors and metabotropic glutamate receptors. In the following presentation, I will outline how GPCRs interact with a wide variety of unexpected intracellular signaling proteins. Furthermore, I will demonstrate that different GPCRs can regulate identical intracellular signaling pathways, via distinct mechanisms to achieve entirely different responses. I will show that GPCR activation can lead to the spatial-temporal patterning of second messenger regulated protein kinases and that signal transduction is not only regulated by the activity-state of a signaling protein but also by its spatial localization within a cell.

VIb Studying Chemistry Inside the Living Cell: Proteins and their Interactions

Vali Raicu^{1,2}, David B. Jansma², James D. Friesen², R. J. Dwayne Miller¹ (¹ *Departments of Chemistry and Physics, and* ² *Banting and Best Department of Medical Research, University of Toronto*)

Forster resonance energy transfer, also known as fluorescence resonance energy transfer (FRET), is a process through which the excitation of a fluorescent molecule is transferred nonradiatively to a nearby molecule. We introduce a microspectroscopic method, based on FRET measurements and analysis, for determination of the fraction of a protein population present in protein complexes (i.e., the stoichiometry of protein complexes) in single living cells. The method relies on full spectral analysis of the fluorescence signature to extract the efficiency of donor/acceptor energy transfer, and a theoretical model introduced by us, which relates the average efficiency of donor/acceptor energy transfer in protein populations (or the apparent efficiency, E_{app}) to the energy transferred in a single donor/acceptor pair (E , the true FRET efficiency). We applied our method to microscopic investigations of complex formation of the Ste2 a-factor-receptor protein (Ste2p), labeled with green fluorescent protein (GFP), cyan fluorescent protein (CFP), and yellow fluorescent protein (YFP), in living yeast cells (*Saccharomyces cerevisiae*). By measuring E_{app} for two donor/acceptor pairs, Ste2p-CFP/Ste2p-YFP and Ste2p-GFP/Ste2p-YFP, we are able to determine E and, subsequently, the fraction of interacting Ste2 proteins, which approaches 100%. Ste2p is a member of a large family of proteins, called G protein-coupled receptors (GPCRs), which represent the largest class of targets for drugs. Our observation of total dimerization of Ste2p may have important implications in drug design, as the current drugs directed at GPCRs have been almost exclusively designed by assuming that these receptors are monomeric.

VIc **Endocytosis of BMP receptors is mediated by clathrin coated pits**

Shayamala Thinakaran, Luna Rahman , Harshan Abeyagoonasekara, Anja Nohe, Nils Petersen (*Department of Chemistry, University of Western Ontario*)

Bone Morphogenetic Proteins (BMPs) play important roles during embryonic development and especially in chondrogenesis, osteogenesis, neurogenesis and hematopoiesis. Bone morphogenetic protein (BMP) receptors have been shown to induce a number of cellular events. The initiation of the signal transduction starts by the binding of a BMP to a corresponding set of BMP receptors leading to the activation of Smad and p38 pathway. The signaling cascade, both initiation and termination, of bone morphogenetic receptors (BMP) has not been fully elucidated. Our study examines the interaction of these BMP receptors (type I: BrIa and type II: BRII) with the trimeric adaptor protein, AP-2. The AP-2 is an essential component of clathrin-mediated endocytosis, complexing clathrin proteins to the plasma membrane during the process of endocytosis. Western blot analysis has shown that the beta-2 subunit of AP-2 does bind to BMP type Ia and II receptors in mammary cancer cells (A431). In order to examine the in-vivo co-localization of the receptors with AP-2, Image Cross Correlation Spectroscopy (ICCS) and Fluorescence Resonance Energy Transfer (FRET) studies were performed on the membrane of A431 cells. ICCS is a powerful tool to examine the percent of co-localization of two proteins on the cell membrane. Using this technique our results indicate that the receptors do co-localize with beta-2 subunit of AP-2 to a high content. Upon stimulation with BMP-2, the co-localization and interaction of AP-2 with BRIa increases while that of the BRII remains relatively same. FRET is a powerful tool to investigate protein interaction which take place within 10-100nm. We used this to investigate if the interaction of the BMP receptors with AP-2 beta takes place directly on the cell surface. Our FRET analyses confirmed that BRIa and BRII do interact with beta-2 subunit directly on the cell surface and showed the same pattern than the ICCS. Upon BMP-2 stimulation FRET increases between BRIa and AP-2 whereas FRET between BRII and AP-2 is not significantly changed. These results attest to the hypothesis that BMP receptor signaling events may be initiated via the clathrin-mediated endocytosis through the interaction of AP-2 with BRIa and BRII.

VIId **Differentiation of structures within single cardiomyocytes based on non-linear multimodal microscopy**

Catherine A. Greenhalgh (a), Juerg Aus-der-Au (b), Jeff Squier (b), Steve Elmore (c), Hans van Beek (c), Virginijus Barzda (a). (a) *University of Toronto*; b) *Colorado School of Mines*; c) *Free University Amsterdam*)

The simultaneous detection of second and third harmonic generation (SHG and THG) and multiphoton excitation fluorescence (FL) from the same focal volume have led us to the development of a multimodal imaging tool. By employing the different contrast mechanisms, differentiation of structures inside single adult rat cardiomyocytes can be achieved with our microscope. Based on FL labelling of the mitochondria with TMRM dye and morphology we were able to assign the structure revealed by THG to the mitochondria. Results showed that SHG was anti-correlated with THG and possessed the characteristic pattern of the myofibrils in the myocyte in accordance with literature. The hypercontraction of the myocyte showed that the SHG signal originates from the non-centrosymmetric crystalline structure rather than from molecules in the myofibrils that have a high second order non-linear susceptibility. Unlike FL, SHG and THG signals do not degrade over time. Preliminary analysis showed changes in THG signals associated with functional activity of mitochondria. We have demonstrated that with this technique, one can investigate the physiological dynamics of structures within a single cell.

VIIa **Elasticity of Composite Actin Networks**

David Weitz (*Department of Physics, Harvard University*)

The rheology of actin networks formed in the presence of actin binding proteins, which both cross-link and bundle the actin, exhibits a remarkable range of behavior. This talk summarizes this behavior, and suggests a means of parameterizing the results with a state-diagram. It also shows how the network properties can be understood, in some important instances, in terms of the single filament properties.

VIIb **Enhanced conformational sampling via novel variable transformations and adiabatic dynamics: Applications to small proteins and drug binding in the HIV-1 protease**

Mark E. Tuckerman (*New York University*)

One of the computational grand challenge problems is the development of methodology capable of sampling conformational equilibria in systems with rough energy landscapes. If met, many important problems, most notably protein folding, could be significantly impacted. In this talk, a new approach will be presented in which molecular dynamics is combined with a novel variable transformation designed to warp configuration space in such a way that barriers are reduced and attractive basins stretched. The new method rigorously preserves equilibrium properties while leading to very large enhancements in sampling efficiency. Moreover, when variable transformations are combined with an adiabatic separation between a reactive subspace of coordinates and the remaining degrees of freedom, an efficient method for exploring free energy surfaces emerges. These new methods are applied to study the conformational equilibria of small proteins and complexes of the HIV-1 protease with fullerene-derived inhibitors. In the latter example, a possible mechanism for increasing the binding affinity in the active site of the HIV-1 protease is revealed.

VIIc **Analysis of the Adsorption Behaviour of Mandelate Racemase and its Substrate: Comparison of Chronocoulometry and EQCN Techniques**

Christa L. Brosseau, Sharon G. Roscoe (*Acadia University*)

The adsorption behaviour of the enzyme mandelate racemase and its substrate, mandelic acid, has been studied at the platinum and gold polycrystalline surfaces using the electrochemical quartz crystal nanobalance (EQCN) and chronocoulometry techniques. These techniques are also compared directly with cyclic voltammetry measurements made concurrently in situ in the phosphate buffer solution, pH 7.4. These measurements provide complementary results important for the interpretation of the adsorption processes with EQCN measurements. The adsorption behaviour of these molecules differs substantially with the two surfaces. The adsorption behaviour is strongly influenced by the electrocatalytic oxide film that develops at anodic potentials on the platinum surface. On the other hand, gold which is essentially devoid of oxide over the investigated potentials shows a much smaller response to adsorption by protein compared with that of the substrate molecule. For both surfaces, the surface concentrations and Gibbs free energy of adsorption were determined. The results indicate that with proper care, the EQCN technique provides information on the adsorption behaviour of proteins on these surfaces.

VIIId **Probing Gene Expression and Regulation at the Single Molecule Level**

Michelle D. Wang (*Department of Physics, Cornell University*)

Biological organisms must compactly store and yet efficiently read the huge amounts of genetic information contained in their DNA. Many DNA-based enzymes involved in these processes function as highly processive molecular motors capable of translocating over thousands of base pairs without detaching from the DNA template. These motors face mechanical obstacles to their movement, especially in the highly packed DNA of chromatin, and many of these obstacles are known to be important regulators of gene expression. I will discuss our recent progress towards understanding the mechanical constraints of gene expression using single-molecule studies.

Poster Presentation Abstracts

P1 **Autoactivation, activation, repression – mathematical model for Pax-5 transcription.**

Miroslava Cuperlovic-Culf, Rodney J. Ouellette (*Beauséjour Medical Research Institute*)

Transcription factor BSAP binds to promoters of various genes acting both as a transcription activator and as a repressor in a concentration dependent manner. Here we present an analysis of the promoter sequences of Pax-5, CD19 and IgJ in a search of the Pax-5 binding sites for activation/repression. This analysis shows that Pax-5 may also be anticipated to show autoactivation. Using these results we present a preliminary attempt to mathematically model the expression of Pax-5 including the possibility for autoactivation keeping in mind its role in activation and repression in terms of concentration and promoter binding sites affinity. The properties of the Pax-5 gene and protein were determined using bioinformatic methods. The model is being developed using classical biochemical kinetics with protein and mRNA concentrations represented as a set of nonlinear differential equations.

P2 Analysis of the composition of AP2 complex

Luna Rahman, Shamayala Thinakaran, Harshan abeyagoonasekara, Anja Nohe, Nils Petersen (*Master's candidate*)

ANALYSIS OF THE COMPOSITION OF AP2 COMPLEX

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The coated pits in the plasma membrane consists of the Clathrin triskelion and the adaptor proteins (AP). Among the four identified adaptor proteins AP2 is known to play the major role in clathrin mediated endocytosis. It consists of two heavy subunits (alpha: AP2- α ; and beta: AP2- β ;) and a medium γ subunit and a small δ unit. Up to now it was thought that AP-2 can only exist as a heterotetrameric complex composed of all four subunits. However, previous studies showed that the alpha subunit of AP2 binds to the tyrosin kinase receptor and the beta subunit binds through the serine threonine receptor, which raises the question whether these two subunits of AP2 are always in the tetrameric complex or not. Image Correlation Spectroscopy (ICS) and Image Cross Correlation Spectroscopy (ICCS) are powerful techniques, which are developed in our lab to study the distribution of proteins on the cell membrane. The advantage of these techniques is that, calculation using data from the high magnification confocal images of different proteins of interest labeled fluorescently can give us not only qualitative but also quantitative information about the distribution these proteins. We used these techniques to localize the two subunits of AP2- α ; and AP2- β ; and calculate the percent of colocalisation of these two proteins labeled with fluorescent probe of different colors in fixed A431 cells.

Preliminary studies based on ICCS showed that the alpha and the beta subunit of AP2 do not colocalize to 100%. The localization of alpha and beta subunits of the adaptor protein, when they are the bound together is a question of interest. Both the subunits are known to bind to clathrin and to receptor proteins during endocytosis. Our data give evidence that the AP-2 complex is very dynamic and flexible and that the alpha and the beta subunit of AP2 might move separately in the plasma membrane.

P3 **Volumetric Characterization of Homopolymeric Amino Acids**

Nicolas Taulier, Gholamreza D. Noudeh, Tigran V. Chalikian (*Faculty of Pharmacy, University of Toronto*)

We have determined the partial molar volumes, expansibilities, and adiabatic compressibilities for poly(L-alanine), poly(L-proline), and poly(L-threonine) within the temperature range of 18–55°C. In addition, we have determined at 25°C changes in volume, ΔV , and adiabatic compressibility, ΔK_S , associated with the coil-to-helix transitions of poly(L-lysine) and poly(L-glutamic acid) and the α -to- β transition of poly(L-lysine). We have interpreted our volumetric data as suggesting that poly(L-alanine) and poly(L-proline) are not fully unfolded and, probably, retain some solvent-inaccessible core. Further, we propose that poly(L-threonine) is fully unfolded with the majority of its atomic groups being solvent-exposed. Near zero changes in volume and compressibility accompanying the coil-to-helix transitions of poly(L-lysine) and poly(L-glutamic acid) suggest that the coil-to-helix transitions of the polypeptides do not result in any significant enhancement of solute hydration. By contrast, the α -to- β transition of poly(L-lysine) causes slight but statistically significant increases in volume and compressibility, an observation that may suggest that the β -sheet conformation of poly(L-lysine) is slightly less hydrated than its α -helical conformation. In general, our results provide a quantitative volumetric description of the hydration properties of the homopolymeric polypeptides investigated.

P4 **Amphotericin B interactions with a supported lipid monolayer under electrochemical control – A Fluorescence Resonance Energy Transfer study**

Robin Stoodley, Dan Bizzotto (*Advanced Materials and Process Engineering Laboratory, Chemistry Dept, UBC*)

The interaction of Amphotericin B, an anti-fungal drug, with a supported lipid monolayer is being investigated using fluorescence resonance energy transfer (FRET) between diphenylhexatriene (DPH)-tagged lipid in the monolayer and Amphotericin B itself. The lipid monolayer is supported by adsorption onto a hanging mercury drop electrode, allowing potential control over the system, akin to transmembrane potential. Amphotericin B is introduced to the electrolyte and can thus freely interact with the monolayer both at the air-solution interface and at the electrode-solution interface. It has been earlier shown that the interaction of Amphotericin B with the supported monolayer is dependent on the applied potential at the electrode-monolayer interface. Because FRET between the drug and fluorescent-tagged lipid is highly sensitive to the separation distance between the two, we hope to use this method to probe the potential-dependence of Amphotericin B intercalation into the monolayer.

(This work is currently in progress)

P5 Probing Hydration of Counterions in the Vicinity of Nucleic Acids

Anna Tikhomirova, Tigran V. Chalikian (*Faculty of Pharmacy, University of Toronto*)

This study is aimed at investigating the hydration properties of univalent counterions in the vicinity of nucleic acids. We use densimetric and ultrasonic velocimetric measurements to evaluate the partial molar volumes and adiabatic compressibilities of the poly(rA)poly(rU), poly(dAT)poly(dAT), and poly(dIdC)poly(dIdC) polymeric duplexes, and the poly(rU) single stranded polymer in the presence of LiCl, NaCl, KCl, RbCl, CsCl, NH₄Cl and N(CH₃)₄Cl. Prior to experiments, each polymeric DNA or RNA was dialyzed against a pH 6.7 buffer consisting of 0.1 mM cacodylic acid, 0.1 mM EDTA, and 50 mM of the corresponding salt. When determining the partial molar volume and adiabatic compressibility of the nucleic acids, we have taken into account the difference in salt concentration between the DNA solution and the buffer which is due to the Donnan equilibrium. If counterions around DNA are independently hydrated, the partial molar volume/adiabatic compressibility of a nucleic structure in each salt plotted versus the partial molar volume/adiabatic compressibility of the salt should yield a straight line with a slope of unity. Our results reveal that nucleic acids influence the hydration properties of counterions. Significantly, hydration of a counterion may increase or decrease in a manner that depends on the type of the counterion and is virtually independent of the type of nucleic acid structure. Our results have implications for the thermodynamics of processes that involve nucleic acids and are accompanied by release/uptake of counterions. Such processes include helix-to-helix and helix-to-coil transitions of nucleic acids as well as drug-DNA and protein-DNA interactions.

P6 Anesthetic-enhanced Membrane Fusion Examined Using Two-photon Fluorescence Cross – correlation Spectroscopy

Jody L. Swift, Anna Carnini, Tanya Dahms, and David Cramb (*University of Calgary*)

Vesicle fusion has been used as a model for viral fusion with cells, but measurement of the kinetics of the process simultaneously with vesicle size has been elusive. In this study, we describe the use of two-photon excitation fluorescence cross correlation spectroscopy (TPE-XCS) to measure simultaneously the kinetics and structural changes occurring early in the process of vesicle fusion. We have shown that in the presence of a novel fusion agent, halothane, unilamellar vesicles of dioleoylphosphatidylcholine fuse at rates smaller than the diffusion limit. We postulate that halothane works as a gentle perturbation agent by increasing the disorder in the phospholipid bilayer. Moreover, under the conditions used in the present study, appears to be complete when approximately 6 vesicles have fused.

P7 Lateral Phase Separation in Mixed Monolayers of Dipalmitoylphosphatidylcholine and Dilauroylphosphatidylcholine

Jacqueline Sanchez, Antonella Badia (*Universite de Montreal*)

Brewster angle microscopy (BAM) and atomic force microscopy (AFM) imaging have been used to investigate domain formation in dipalmitoylphosphatidylcholine (DPPC)/dilauroylphosphatidylcholine (DLPC) monolayers, a mixture where phase separation arises from a difference in the alkyl chain length of the lipid components. The condensed domain structure (shape and size) was found to depend on both the mole fraction of DPPC and the surface pressure. DPPC and DLPC are miscible in the liquid-expanded phase at low surface pressure. At a surface pressure of 32 mN/m, phase separation into condensed DPPC-rich domains and a fluid DLPC matrix results. Between 32 and 40 mN/m, a drastic change in the film morphology occurs, resulting in a distortion of the DPPC domain shape. The phase behavior of the DPPC/DLPC monolayers will be discussed.

P8 Anomalous Diffusion: An effect of crowding on protein diffusion

Daniel Banks, Cecile Fradin (*McMaster University*)

One of the cell's features which adds to its great complexity is its extremely high concentration of macromolecules (up to 400 g/L). For practical reasons, many in vitro experiments are done in dilute solution, effectively ignoring the effects of macromolecular crowding. Interesting effects of crowding have already been found, which provides motivation for this study to determine whether the anomalous diffusion found in the cytoplasm is another effect of crowding. This study uses FCS (Fluorescence Correlation Spectroscopy) to probe systems modeling the crowded cytoplasm. The results suggest that some proteins diffuse anomalously on the millisecond time scale under crowded conditions.

P9 The Effect of High Pressure on the Stability of DNA

Gamal Rayan, Tigran V. Chalikian, Robert B. Macgregor Jr. (*Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto*)

We are studying the effect of high hydrostatic pressure on the stability of DNA to understand the role of hydration in the stabilization of the DNA double helix. Data obtained from this study are useful for understanding the thermodynamics of these polymers. Spectroscopically monitored thermal and pressure induced denaturation was employed in order to acquire the results. The molar volume change of the helix to coil transition (δV) of poly [d(A-T)] changed from positive (for polymers with melting temperature greater than ~ 50 °C) to negative (for polymers whose melting temperatures are lower than ~ 50 °C). The observed results were in agreement with a phase diagram for the helix to coil transition of double stranded nucleic acids. This is a significant step in demonstrating the general validity of the phase diagram. The cooperative length measured by pressure induced transition is much larger than the one measured by heat induced transition. The structural origin of this difference is still under investigation.

P10 **Mixed Phospholipid Bilayers: Efficient Tools for Nanopatterning**

Patricia Moraille, Antonella Badia. (*Department of chemistry, Université de Montréal*)

We have developed a novel method to create extensive parallel striped patterns in phospholipid bilayers. The stripes (150-250 nm in width) are generated by the Langmuir-Blodgett (symmetric and asymmetric) transfer of phase-separated binary phospholipid mixtures onto mica. The patterns generated are widely accessible in terms of lipid composition, surface pressure, and film deposition conditions. They are also compatible with further treatments such as fluid phase extraction with a non ionic detergent. An atomic force microscopy study of these nanostructured films demonstrates their application as tools for spatially directing the deposition of proteins and biomolecules onto different regions of the surface. Such striped bilayer architectures combined with the introduction of different headgroup-functionalized phospholipids constitute a new generation of water stable templating tools for nanopatterning applications.

P11 **Spectral tuning of tetrapyrroles in photosynthetic light harvesting**

Alexander B. Doust, Gregory D. Scholes, Christopher N.J. Marai, Delmar S. Larsen (*Dept of Chemistry, Univeristy of Toronto*)

Linear oligopyrroles are an important class of pigments found in numerous marine photosynthetic light-harvesting antenna biliproteins, whose utility is to absorb light in the visible part of the spectrum where chlorophyll and carotenoid absorption is minimal. These biliproteins have not been as well characterised as other chlorophyll- and caroteinoid-containing light harvesting proteins. Phycoerythrin 545, isolated from a unicellular photosynthetic cryptophyte alga *Rhodomonas CS24*, is a prime example of such a biliprotein. The three chemically distinct open-chain tetrapyrrole pigment types found in this biliprotein have unique optical properties, which ensure the fast and efficient harvesting and transduction of excitation energy.

High-resolution x-ray crystallographic data of phycoerythrin 545, coupled with absorption, emission and polarized steady state spectroscopy, as well as ultrafast non-linear spectroscopies with 20 fs time resolution, will be presented to explain the role of the pigments in the function of the protein. Concurrently, an understanding of the spectral properties of the chromophores will be elucidated by calculations of the excited state energies and oscillator strengths using ZINDO/S, CIS, and TDDFT. The effects of the protein environment on the spectral properties of these tetrapyrrole pigments will be demonstrated to be very important and worth trying to understand.

P12 Slip at Multiple Interfaces on an Acoustic Wave Device in Liquid

Jonathan S. Ellis¹, Gordon L. Hayward², Michael Thompson³ (1) *Institute of Biomaterials and Biomedical Engineering, University of Toronto*; 2) *School of Engineering, University of Guelph*; 3) *Department of Chemistry, University of Toronto*)

Planar slip at multiple interfaces of a transverse-shear mode acoustic wave device is used to explain non-classical results obtained recently in our lab. A four-layer model is used, including the quartz substrate, the gold electrode, a sensing monolayer, and an infinite liquid. Slip is included at the gold-monolayer (inner slip) and monolayer-liquid (outer slip) interfaces. Gold-monolayer slip is interpreted as a stochastic decoupling due to the lability of the Au-S thiol bond, and is modeled with a viscoelastic relaxation time. Changes in both the storage and dissipation at the interface can lead to changes in coupling. Outer slip is modeled as a slip length, commonly used in hydrodynamics. The results of the model, over large ranges of inner and outer slip, produce all four combinations of f_s and R_m increases and decreases. In fact, in the range examined, inner slip alone is sufficient to produce all four combinations. These numerical results are compared to a model system explored in our laboratory involving alkane- and alkynethiol monolayers chemisorbed on an acoustic wave device. Both monolayers were exposed to UV light, which is expected to cause changes in coupling at the monolayer-gold interface. Slip and coupling changes at one or both interfaces can be used to explain the observed experimental results.

P13 Controlling architecture in bio-inorganic composite materials

Erika F. Merschrod S., Peter A. W. Collins (*Department of Chemistry, Memorial University*)

Our research involves the preparation and characterization of bio-inorganic composite materials, emphasizing patterning across length scales. The growth and structure of these new materials serve as models for analogous processes *in vivo* such as bone development or arterial hardening. Herein we present preliminary results on a protein-mineral composite material produced by co-precipitation of apatite and collagen. The material structure is analyzed with powder x-ray diffraction and electron microscopy.

P14 **The Link Between Stress, Patch Capacitance and Mechanosensitive Channel Function Probed with MSC Blockers and Activators**

Fred Sachs, Tom Suchyna (*SUNY Buffalo*)

Patch tension generated by pipette pressure changes, can be assessed by monitoring patch capacitance (C_p) during mechanosensitive channel (MSC) recordings. The cytoskeleton serves to buffer the rate of membrane tension change and modifies the activation and inactivation properties of MSCs. Cytoskeletal disruption by either mechanical or chemical means leads to a larger, more rapid change in C_p (ΔC_p) when a pressure step is applied compared to normal cell-attached patches. We are currently assessing the characteristics of ΔC_p for patches formed from pure lipid bilayers. In order to compare ΔC_p measurements from patches with varying configurations (outside-out vs cell-attached) and composition (cellular membranes vs synthetic bilayers) we must know the total patch capacitance. However, it is difficult to determine the total patch capacitance due to the contributions of the seal region. We have shown that pressure can generate electroosmotic potentials in symmetrical KCl solutions, and that the seal leak is cation selective. The sealed membrane may contribute a significant fraction of the total patch capacitance as we have shown by modeling the seal-glass annular interface as a core conductor. Many agents that modify MSC gating properties appear to act at the interface of the membrane and the channel. We have examined how various MSC modifying agents affect membrane mechanics by measuring their effects on membrane capacitance. Gd^{3+} (a blocker) and Ca^{2+} (which increases the rate of channel activation) perfused onto outside-out patches reduce the resting patch capacitance, and increase seal resistance. However, they do not affect C_p during a pressure step, i.e. they apparently don't affect membrane stiffness significantly. However, Gd^{3+} is nearly 100 fold more potent than Ca^{2+} on reducing resting patch capacitance. Lysophosphatidylcholine-myristoyl, which activates the bacterial MscL channel at $2 \mu M$, and genistein, which activates gramicidin at $5 \mu M$, have little effect on eukaryotic MSCs, and produce a weak decrease in resting capacitance at $10 \mu M$. GsMTx-4, a hydrophobic peptide with a net charge of +5 that blocks MSCs in rat astrocytes at $5 \mu M$, had no effect on patch capacitance. It may be that GsMTx-4 and LPC act locally on membranes near the channels – the boundary lipids – but a region too small to be seen by membrane capacitance.

P15 Structural Stability of Monodisperse Species of DNA Frayed Wires

Rashid M. Abu-Ghazalah, Robert B. Macgregor Jr. (*Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto*)

Oligodeoxynucleotides containing long, consecutive, terminal guanosine residues ($d(N_xG_y)$ or $d(G_yN_x)$, $x \geq 5$, $y \geq 12$), self-aggregate to form a set of structures termed DNA frayed wires. To date, all chemical, physical, and biological experimental procedures were applied on polydisperse samples of frayed wires. Elucidation of the structure of frayed wires and the stability and molecular mechanisms leading to their formation will be feasible only with monodisperse samples. Therefore, the ability to separate the different species of frayed wires based on size and then to extract them became essential. Polyacrylamide gel electrophoresis was employed for the first part, while electro-elution and/or passive diffusion were used for collecting the DNA. We investigated the structural integrity of isolated monodisperse species of frayed wires arising from $d(A15G15)$. All species exhibited remarkable thermostability and retained their structural integrity without redistributing into a set of polydisperse structures after incubation at 100°C . Furthermore, the incubation of monodisperse species of frayed wires with the parent strands, $d(A15G15)$, did not lead to a change in the size of the isolated DNA samples.

P16 Ran-GTP/Ran-GDP Gradient across the Nuclear Membrane

Asmahan A. Abu-Arish, Cecile Fradin (*McMaster University, Department of Physics & Astronomy*)

The GTP-binding protein Ran is the major regulator of nucleo-cytoplasmic transport across the nuclear pore complex (NPC). It has been suspected for a long time that there is a gradient from Ran-GDP to Ran-GTP when going from the cytoplasm to the nucleus, and that this gradient drives both nuclear import and export. Characterizing the Ran-GTP/Ran-GDP gradient across the NPC will hopefully help understanding the nuclear transport mechanism, and I intend to study that. For that purpose, a fluorescently-labeled biological probe (YRC) has been constructed to detect the presence of Ran-GTP by binding to it in an extended conformation. Fluorescence Correlation Spectroscopy (FCS) was used to measure the local concentrations and detect the dynamics of the conformational changes of the probe in the presence/absence of Ran-GTP/Ran-GDP.

P17 Photochromic Liposomes: Incorporating Supramolecular Function

R. Scott Murphy, Yili Bai, Kristen Louis (*University of Regina*)

Supramolecular chemistry is an ambitious goal, yet well recognized, avenue of chemistry with potential benefits to many disciplines. Supramolecular assemblies are more commonly being used to custom-tailor functions in areas such as drug delivery, chromatography, and asymmetric synthesis. The functionality achieved by these assemblies is a consequence of their supramolecular structure. A recent focus of our research program has been on the design, synthesis, and photophysics of photochromic liposomes. We have synthesized two photochromic compounds that will be incorporated into the lipid bilayer of phosphatidylcholine liposomes as a strategy for controlling membrane permeability. The first compound is a dithienylethene derivative, which displays high thermal irreversibility, and will provide absolute photocontrol over membrane permeability. The photochromism of dithienylethenes has been studied in single crystals, and more recently in polymer films, and liquid crystals, but *has never been examined* in lipid bilayers. The second photochromic molecule is a *novel* spirooxazine derivative, which contains long saturated alkoxy substituents so as to improve lipid complementarity. We anticipate that the photoinduced isomerization of these molecules will cause a reorganization of the local lipid order to alter its permeability. To better evaluate the effect of integrating photochromic compounds in a lipid bilayer, we are currently employing photophysical probe molecules that report on membrane structure, and permeability. These studies will lead to the development of photochromes with improved lipid complementarity, and supramolecular function. The design and synthesis of our photochromic compounds will be presented, and preliminary photophysics on their incorporation into liposomes will be discussed.

P18 Interaction Between Focused Laser Beam and Red Blood Cell Membrane

Felix H.C. Wong, Cecile Fradin (*McMaster University*)

In order to study red blood cell membrane fluctuation with fluorescence correlation spectroscopy (FCS), we need to obtain correlation function by exciting fluorescent particles diffusing close to the membrane with a focused laser. However, a damaging process to the red blood cell was observed during the experiment. First, the laser leaves a small imprint on the red blood cell membrane in the shape of a crater. Then, the cell loses its biconcave shape, gradually acquiring a spherical shape, and eventually the cell bursts. The final destruction of the red blood cell is well understood in terms of creations of free radical from the fluorescent species, and is the process exploited in photodynamic therapy. The time need to kill the cell was determined to depend on laser input intensity, fluorophore concentration in the sample, size of the laser focal volume, and wavelength of the laser. While we observed that the time necessary to form the crater is independent of fluorophore concentration but depends on the laser input intensity.

P19 **2D-IR Spectroscopy of Water**

R. J. Dwayne Miller¹, Barry Bruner¹, Michael L. Cowan¹, Nils Huse², T. Elsaesser², Erik T. J. Nibbering² (1) *Departments of Physics and Chemistry, University of Toronto*; 2) *Max Born Institute for Nonlinear Optics and Short Pulse Spectroscopy*)

Hydrogen bonding gives water many of its unique and fascinating properties, and is essential to the biological function of proteins and thus life. Using phase-locked sequences of sub-100 fs, 3 micron pulses we have performed 2D-vibrational spectroscopy, an optical analogue to 2D-NMR spectroscopy, on the O-H vibrational stretch mode in samples with different ratios of heavy water and water. By probing the coupling between neighboring O-H vibrational modes, we have shed light on the dynamics and the nature of the hydrogen bond network in water.

P20 **Canine Plasminogen and ANS: An investigation into the nature of binding between a fluorescent probe and a serum protein**

David M Carter, Jack Kornblatt (*Dept. of Chemistry and Biochemistry Concordia University*)

Plasminogen is a proteolytic zymogen, whose primary function involves dissolution of the fibrin based blood clot. (1) The protein exists in two distinct conformations; an inactive compact closed form and an inactive opened form. (2) Activation results from the cleavage of an Arg-Val bond with concomitant formation of an active site typical among the serine proteases. The canine variant of plasminogen (DPgn) was shown to bind the fluorescent dye 8-anilino-1-naphthalene sulfonate (ANS). Binding was pH dependent with tighter binding exhibited at low pH. Native gel electrophoresis was used as a qualitative means to establish the presence of binding. At pH 6.5 binding was not observed in gels stained with ANS. In contrast, binding could be distinguished at pH 3.3 as fluorescent bands when viewed on a UV transilluminator. Coomassie stains of the same gels yielded identical staining patterns, indicating that DPgn in fact bound ANS. Steady-state fluorescence titrations were conducted in an attempt to characterize ANS binding affinity. Binding isotherms indicated no saturation with ANS concentrations as high as 50 μM , a concentration where inner filter effects became substantial. Though saturation was not evident, the isotherms were not linear and a model invoking two binding sites was proposed. This model assumes one binding site with low fluorescence yield and another with high yield. Isothermal titration calorimetry was used for validation of the two site model. At pH 2.9 the isotherms thus generated fit this model well. The data indicated a high affinity site with an associated $K_d = 9 \mu\text{M}$ and a weaker affinity site with an associated K_d approaching 50 μM . At pH 4.1 the binding data could not be reliably fit to any model; an observation in agreement with the establishment of a pH dependence upon binding. To answer the question, of whether pH induces binding as a result of increased protein flexibility, analytical ultracentrifugation was employed. Sedimentation velocity experiments indicated that the frictional ratio (f/f_0) of DPgn does not appreciably differ with pH. At pH 6.3 a f/f_0 ratio of was 1.6 was observed whereas the ration decreased to 1.5 at pH 3.4. This suggests that pH does not enhance binding as a result of greater protein flexibility, but rather as a protonation event occuring at or near the ANS binding sites. Future experiments will further establish the role pH plays in DPgn hydrodynamics as it pertains to the binding of ANS.

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P21 **Calculation of Couplings and Energy-Transfer Pathway between Chromophores of Phycoerythrin 545 in Rhodomonas Cs24 by the ab initio and TDDFT Transition Density Cube method.**

Christopher N. J. Marai, Alexander B. Doust, Gregory D. Scholes (*Department of Chemistry, University of Toronto*)

The crystal structure of Phycoerythrin 545 protein in the *Rhodomonas Cs24* a unicellular photosynthetic algae has recently been determined to 0.97Å resolution. The protein consists of eight linear open-chain tetrapyrroles chromophores linked to the protein. Each chromophore has unique excited-state properties that are modified by interactions with the apo-protein. A theoretical elucidation of the chromophore couplings and energetics that govern the dynamics in the light-harvesting apparatus of *Rhodomonas Cs24* is reported. Evaluation of the Coulombic contribution to the coupling among chromophores was investigated by the Transition Density Cube method, a generalization of Förster's theory. Transition densities of donors and acceptors were calculated by CI-singles and Time-Dependent DFT wavefunctions. Excited state calculations reveal that the 8 chromophores are at least of two distinct chemical species. Moreover results are supportive of the assertion that the surrounding protein and solvent environment plays a role in excited state properties. Further efforts are underway to model precise contributions that the protein 'scaffold' and solvent environment make to the excited state properties of the chromophores.

P22 **Atomic force microscopy investigation of the effects of fixation on cellular mechanical properties**

Jeffrey L. Hutter, Bosco Chan, Jie Chen, Shashi Uniyal, W.K. Wan (*The University of Western Ontario*)

The atomic force microscope (AFM) has provided nanoscale analyses of surfaces of cells that exhibit strong adhesive and cell spreading properties. However, it is frequently reported that prior fixation is required for reliable imaging of cells with lower adhesive properties. We have used the AFM to study the effects of glutaraldehyde fixation on the elastic properties of various cell lines (rhabdomyosarcoma RDX2C2 cells and NIH 3T3 fibroblasts) deposited on different substrates (bare and collagen-coated silicon and plastic surfaces). We find a sharp increase in the Young's modulus for light fixation, saturating at a tenfold increase at high fixative concentrations. RDX2C2 cells showed no apparent microstructure in AFM images, although F-actin filaments are detected by fluorescence microscopy. This is in sharp contrast with 3T3 cells, in which F-actin filaments are detected via both microscopies as filamentary structures. This may due to differing organization of the F-actin filaments within the two cell lines.

P23 **The role of clustering in network dynamics**

Patrick McGraw, Michael Menzinger (*Chemistry Department, University of Toronto*)

Recent interest in the structure and dynamics of complex networks has used a number of models to mimic the structure of real social, technological and biological networks. Much interest has been focused on the distribution of degree, or number of connections per node, and the frequent occurrence of scale-free structure. The 'small-world' property of short path length has also been frequently noted. But another important property of many real complex networks is that of *clustering*, or the occurrence of more triangles and other loops than would occur by chance. Many recent network models have failed to take clustering explicitly into account, and only recently have any efforts been made to separate the effects of clustering from those of degree distribution. We examine the effects of clustering on the dynamics of networks; in particular, the basin of attraction sizes in Hopfield neural networks, and the synchronizability of networks of oscillators.

P24 **The Chicken Chorioallantoic Membrane as a Model for Two-Photon Photodynamic Therapy of Age-Related Macular Degeneration**

Kimberley S. Samkoe, David T. Cramb (*Department of Chemistry, University of Calgary*)

Two-photon excitation photodynamic therapy (TPE-PDT) is being investigated as a clinical treatment for age-related macular degeneration (AMD) using the chicken embryo chorioallantoic membrane (CAM) as a model. The CAM is a highly vascularized membrane that develops between Days 3 and 12 of a chicken's gestation period and undergoes period rapid angiogenesis between Days 5 and 12. This angiogenic blood vessel growth is ideal for modeling the choroidal neovascularization that occurs in the wet form of AMD. Existing treatment regimes for AMD, such as one-photon excitation (OPE) PDT, have a tendency to induce further vision loss due to collateral tissue damage in the treatment volume. The development of TPE-PDT has the potential to provide a more specific and therefore advantageous therapy regime. Initially, TPE-PDT was investigated using an ex ovo model system for the CAM. Laser ablation studies were successful in mimicking leaky vessels found in AMD. As well, the partitioning and photochemistry of liposomal Verteporfin were investigated by monitoring the TPE-induced fluorescence of the photosensitizers in order to characterize the drug in vivo. Localization of the photosensitizer appears to be greatest on the upper vessel wall, which indicates a potentially strong treatment locale for TPE-PDT in the CAM. Presently, the study of TPE-PDT is being extended into the in ovo CAM model. Ideally, the in ovo system will overcome the limitations of the ex ovo system while allowing the effects of the TPE-PDT to be monitored over several days.

P25 **Thermodynamics of Hoechst 33258 association with poly(dAdT)poly(dAdT), poly(dA)poly(dT) and d(CGCGAATTCGCG) duplex: Volumetric, calorimetric and spectroscopic studies**

Feixue Han, Tigran V. Chalikian (*Department of Pharmaceutical Sciences, University of Toronto*)

We have used ultrasonic velocimetry, high-precision densimetry, circular dichroism and fluorescence spectroscopy, and isotherm titration calorimetry to characterize the binding of Hoechst 33258 to the poly(dAdT)poly(dAdT) and poly(dA)poly(dT) polymeric duplexes as well as to the d(CGCGAATTCGCG) oligomeric duplex at 25 °C. We report the changes in volume and adiabatic compressibility that accompany the binding of the drug to DNA. In addition, we report the values of the binding free energy, enthalpy, and entropy. Association of Hoechst 33258 with each DNA is accompanied by negative changes in volume and compressibility. Based on our analysis of the macroscopic and microscopic properties of Hoechst 33258 association with DNA, we interpret our volumetric data in terms of the differential hydration properties of DNA structures in their ligand-free and ligand-bound states. Our calorimetric data reveal that each drug-DNA binding event studied in this work is entropy-driven and proceeds with an unfavorable change in enthalpy. The favorable entropy of the binding predominantly results from the binding-induced release of water molecules from the hydration shells of the drug and DNA to the bulk. In general, we discuss the role of hydration in determining the thermodynamics of drug-DNA recognition as reflected in changes in volumetric and calorimetric parameters.

P26 **MOLECULAR BASIS OF PROTON BLOCKAGE IN AQUAPORINS**
NILMADHAB CHAKRABARTI, BENOIT ROUX, REGIS POMES (*HOSPITAL FOR SICK CHILDREN*)

Water-transport channels in membrane proteins of the aquaporin superfamily are impermeable to ions, including H⁺ and OH⁻. We examine the molecular basis for the blockage of proton translocation through the single-file water chain in the pore of a bacterial aquaporin, GlpF. We compute the reversible thermodynamic work for the two complementary steps of the Grothuss 'hop-and-turn relay mechanism: consecutive transfers of H⁺ along the hydrogen-bonded chain (hop) and conformational reorganization of the chain (turn). In the absence of H⁺, the strong preference for the bipolar orientation of water around the two Asn-Pro-Ala (NPA) motifs lining the pore over both unidirectional polarization states of the chain precludes the reorganization of the hydrogen-bonded network. Inversely, the translocation of an excess proton in either direction is opposed by a free-energy barrier centered at the NPA region. Both hop and turn steps of proton translocation are opposed by the electrostatic field of the channel.

P27 **Molecular Dynamics of Protein Hydration Water**

Tyler Luchko, Jack Tuszynski, Michele Peyrard (*University of Alberta*)

Water is required for the function of protein. While protein hydration has been extensively studied, attention has generally been focused on the protein and not on the hydration water itself. In this investigation we use molecular dynamics to build upon previous studies by concentrating on hydration water and how its physical properties relate to changes found in the protein. Of particular interest are long range electrostatic interactions such as polarization and dielectric variability. The structure of the water network and orientation correlations are also considered. These results provide a better understanding of the role of hydration water and can help to create better implicit water models in the future.

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P28 **Trials, tribulations, and successes *en route* to membrane protein structure**

Jan K. Rainey, Brian D. Sykes (*Protein Engineering Network of Centres of Excellence*)

Membrane proteins are notoriously difficult to study. Even given a sufficient quantity, standard protocols and techniques in aqueous solution are often inadequate, if not downright incorrect, to handle and examine these typically hydrophobic, but quite possibly amphiphilic, species. Reductionist studies using membrane protein fragments provide a viable starting point for membrane protein structure. Initially, we are determining the structures of a consistent set of peptides under increasingly physiological conditions. We have started with 'membrane mimetic organic solvents, amenable to standard high-resolution solution state NMR. Structural results from homonuclear NMR experiments of a crucial transmembrane helix of the human Na⁺-H⁺ exchanger isoform I are presented alongside discussion of various issues that have arisen during these studies. This 27 residue peptide contains a Pro-Pro kink along with a third Pro, leading to an interestingly less than ideal helix. We are now moving to micellar studies, which allow a more membrane like environment to be probed in the solution state. In parallel, we are conducting solid state NMR studies with oriented lipid bilayer samples. This will provide a direct comparison of structures obtained in different conditions. Finally, using NMR data alongside disparate biochemical and biophysical data as structural restraints for molecular dynamics simulation we hope to define reliable structures for entire proteins.

P29 Structural insights into the binding of peptide agonists to a chemokine receptor

Olga K. Baryshnikova, Jan K. Rainey, Brian D. Sykes (*PhD student*)

The understanding of ligand-receptor interactions represents a substantial challenge due to the very sparse structural information available for receptors. The structures of the natural ligand SDF-1 α , its N-terminus and two peptide agonists of the CXCR4 receptor were compared to identify structural features that might be implicated in binding to CXCR4. Low temperature NMR was employed to determine peptide structures, which offers the advantage of reducing peptide flexibility and stabilizing peptide structure. The conformation of a ligand in its bound state may be argued to resemble low temperature structures. However, even at lower temperatures, the ensembles of agonist peptide structures were mostly disordered upon superimposition. A few regions that demonstrated a distinct conformational feature shared between all ligands were suspected to be a binding motif and docked into a CXCR4 model, based on the structure of rhodopsin and including some mutagenesis results to restrain extracellular loops. The model of CXCR4 in complex with its ligands allows us to propose binding modes for wild type SDF-1 α N-terminus and peptide agonists of CXCR4. This finding may prove useful in the understanding of structural and functional characteristics of CXCR4 and further drug design.

P30 Bax oligomerization at the membrane of a giant vesicle

Dmitri Satsoura, Brian Leber, David W. Andrews, Cécile Fradin. (*Department of Biochemistry, McMaster University, Hamilton, ON, Canada*)

Pore formation at mitochondria is one of the fundamental processes that regulates cell death (apoptosis). Bcl-2 family proteins are key players in both the regulation and the formation of large pores in the outer mitochondrial membrane. Determining molecular mobility and oligomerization state is essential for understanding the molecular mechanism of action of these proteins. Molecular mobility is expected to decrease when the pro-apoptotic protein Bax interacts with membranes. Oligomerization of Bax is believed to result in the formation of pores in the outer membrane of mitochondria. To address the dynamic behavior of this protein in an artificial membrane, peptide triggered insertion of fluorescently-labeled Bax protein into a membrane of a giant unilamellar vesicle (GUV) was examined. Traditionally, protein-membrane interactions have been studied using small or large unilamellar vesicles. These liposomes have also been used to study pore formation by Bax. GUVs, due to their large size have minimum curvature of the membrane and therefore are better models for mitochondrial membrane than conventionally used vesicles. Also GUVs are suitable for optical microscopy enabling us to apply single-molecule spectroscopy to studies of protein-membrane interactions. We report on the application of fluorescence correlation spectroscopy (FCS) to studies of Bax oligomerization in solution and in GUVs with a lipid composition mimicking that of the outer mitochondrial membrane.

P31 Probing Protein-ligand Association with Free Energy Simulations in Four Dimensions

Tomas Rodinger, Régis Pomès, P. Lynne Howell (*Department of Biochemistry, University of Toronto; Institute of Biomaterials and Biomedical Engineering, University of Toronto; Structural Biology and Biochemistry, The Hospital for Sick Children*)

Novel computational techniques for the calculation of excess chemical potentials in full-atomic systems of biological scale are explored. Simulations are carried out via molecular dynamics, incorporating an imaginary fourth spatial dimension as a computationally efficient means of turning on or turning off interactions. The generality, effectiveness, and reliability of the method are demonstrated for the calculation of the hydration free energy of polar and non-polar molecular solutes, as well as ionic species. The absolute binding free energy of various protein-ligand systems differing in the size, hydration state, and accessibility of the binding pocket, is analyzed. Finally, the extension of the four-dimensional method to applications in the identification of binding sites and to the calculation of relative free energy changes through molecular replacement is discussed.

P32 Calculating Equilibrium Properties from a Statistical Mechanical Model for Hydrogen Bonded Networks of Water Molecules: A novel way to calculate potential of mean force

Jia Ke Sun, Régis Pomès (*Department of Biochemistry, University of Toronto, Structural Biology and Biochemistry, Hospital for Sick Children*)

Calculating the partition function of the single-file water chain embedded in the gramicidin channel is crucial for understanding the distribution of water orientations associated with hydrogen bonds and defects. On the basis of the hypothesis in which each individual water molecule in the sequence has four optional microstates such as \uparrow , \downarrow , \leftarrow , and \rightarrow ; [*Bug in the submission process, I don't know what these should be! -Ed.*], we have developed a novel statistical algorithm to describe the statistical mechanics of linear hydrogen-bonded networks of water molecules. This algorithm has been used to calculate the potential of mean force in the system.

The outline of this algorithm can be described briefly in the following way: The general partition function $Z(N)$ of a N water chain is obtained from the equations $Z(N) = VMN - 1U$ where matrix M is built up by base pairs, which are the arrangement of any two water molecules adopted from the above four optional microstates. Then we build up the corresponding vector V and covector U . Finally, using this equation we can calculate the partition function for a chain of any number of water molecules by varying the value of N . By defining the potential energy of the system as the sum of hydrogen bond and dipole energies for each microstate, we can calculate equilibrium properties of the system. Furthermore, we derive a formula for the classification of dipole moments of water molecules in the sequence, which makes it possible to pick up specific microstates from an enormous number of terms in the partition function. The potential of mean force can then be derived from this formula.

P33 Free Energy Simulation Study of Salt Effects on Hydrophobic Association.

Christopher A. Madill, Tomas Rodinger, Hue Sun Chan, Régis Pomès (*Structural Biology and Biochemistry (Hospital for Sick Children), Department of Biochemistry (University of Toronto)*)

Size and ionic effects on hydrophobic association are explored through free energy simulations to analyze some of the fundamental physical forces underlying protein folding and enzyme/ligand association. The potential of mean force governing the association of non-polar solutes in water is calculated with varying ionic strengths from 0-8M guanidinium hydrochloride. Molecular dynamics simulations are run using both methane and neopentane solutes to gauge size effects on the potential of mean force. A theoretical fourth spatial dimension is used in the simulations to efficiently calculate relative free energy changes between all examined systems.

P34 Proton transfer in mixed quantum-classical systems

Gabriel Hanna, Raymond Kapral (*Chemical Physics Theory Group, Department of Chemistry, University of Toronto*)

Proton transfer is of great general importance to many processes in chemistry and biology. Studies of proton transfer in condensed phases require that one consider the dynamics of quantum systems with a large number of degrees of freedom. However, it is not computationally feasible to perform full quantum mechanical simulations of such systems. Therefore, one is led to consider the dynamics of a quantum subsystem coupled to a classical bath. An approach to studying such a composite system is quantum-classical molecular dynamics (QCMD). The main idea is to treat a few crucial degrees of freedom (e.g. a proton) quantum mechanically and the rest of the system (e.g. a solvent) classically. A simple model for a proton transfer reaction ($AH-B \leftrightarrow A^- - H^+ B$) in a linear hydrogen-bonded complex ($AH-B$) dissolved in a polar liquid solvent will be investigated using QCMD.

P35 Escape of peptides from charged aqueous droplets

Styliani Consta, Minh Tran (*Assistant Professor*)

Molecular simulations are used to understand fragmentation processes of ultra-fine charged liquid droplets that contain water as solvent, ions and a single peptide, the alanine dipeptide. Understanding the behaviour of peptides in small droplets and the fragmentation of charged ultra-fine droplets containing the peptide is of fundamental and practical importance in biochemistry and in experimental electrospray techniques that are used in chemistry and biochemistry for the analysis of macromolecules. These ultra-fine droplets while containing the peptide, evaporate, shrink and disrupt due to the Coulomb repulsion between the ions found in the droplet. We study the structure of the peptide and solvent when the system is neutral; the effect of the ions in the structure of the system; changes in the conformation of the peptide when evaporation of solvent takes place and how the peptide leaves the droplet.

P36 **Response of Internal Dynamics to Hydrophobic Core Substitutions in the Fyn SH3 domain**

Anthony Mittermaier, Lewis E. Kay (*Dept. Biochemistry University of Toronto*)

We have used ^{15}N and ^2H NMR spin relaxation experiments to study the response of backbone and side-chain dynamics when a leucine or valine is substituted for a completely buried phenylalanine residue in the SH3 domain from the Fyn tyrosine kinase. Several residues show differences in the timescales and temperature dependences of internal motions when data for the three proteins are compared. Changes were also observed in the magnitude of dynamics, with the valine, and to a lesser extent leucine mutant showing enhanced flexibility compared to the wild-type protein. The motions of many of the same amide and methyl groups are affected by both mutations, identifying a set of loci where dynamics are sensitive to interactions involving the targeted side-chain. These results show that contacts within the hydrophobic core affect many aspects of internal mobility throughout the Fyn SH3 domain.

P37 **Kinetic and Electrochemical Studies of Spreading DMPC vesicles on Gold Slides Using MAC Mode AFM**

Ming Li, Bruno Pettinger, Jacek Lipkowski (*University of Guelph*)

MAC Mode AFM is employed to investigate the kinetic process of spreading DMPC vesicles on gold (111) terrace made from annealing the gold slide. The ripple phase of single bilayer and double bilayer can be observed at different concentrations. Adsorption, fusion and rupture are observed for both kinds of bilayers with time sequence.

P38 Soft X-Ray Spectromicroscopy of Blood Proteins on Patterned Polymer Substrates

Cynthia Morin, Adam Hitchcock, Rena Cornelius, John Brash, Andreas Scholl, Andrew Doran (*McMaster University, Chemistry Department*)

New quantitative techniques for chemical microanalysis which allow detailed study of protein polymer interactions are required for biomaterial interface optimization. We are interested in identification of possible preferences of first sites of protein attachment to polymers used in blood contact medical applications. We have explored Scanning Transmission X-Ray Microscopy (STXM) and Photoemission Electron Microscopy (PEEM) in this context on two systems. These techniques use near edge X-ray absorption spectroscopy (NEXAFS) for chemical identification. Image sequences recorded throughout the C 1s and N 1s regions are used to generate composition maps by fitting the spectrum at each pixel to spectra of pure reference materials. PEEM provides greater surface sensitivity but can be complicated by high sensitivity to topography. With PEEM we have investigated the surface structure of a copolymer blend of polystyrene (PS) and polymethylmethacrylate (PMMA) substrate¹ and adsorption of human fibrinogen on it². Results from PEEM, STXM and atomic force microscopy (AFM) on fibrinogen adsorbed on these polymer blend surfaces will be presented. X-ray microscopy is carried out at the Advanced Light Source (supported by DoE under contract DE-AC03-76SF00098), supported financially by NSERC (Canada) and the Canada Research Chair program. 1. Morin et al., J. Electron Spectroscopy 121 (2001) 203-224 2. Morin et al., J. Electron Spectroscopy (in press)

P39 Electrochemical and PM-IRRAS Studies of Potential Driven Transformations of Phospholipid Bilayers in Gel-state on a Au (111) Electrode Surface

Xiaomin Bin, Iza Zawisza, Jacek Lipkowski (*University of Guelph*)

Electrochemistry and polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS) has been employed to study the fusion of small unilamellar vesicles of DMPC on a Au (111) electrode surface. The electrochemical results show that the vesicles fuse onto the gold surface at charge densities between $-10\mu\text{C}/\text{cm}^{-2} < \sigma_M < 10\mu\text{C}/\text{cm}^{-2}$ to form bilayer where the differential capacity of the film reach the minimum values. When $\sigma_M < -10\mu\text{C}/\text{cm}^{-2}$, the film is detached from the surface but it remains in a close proximity to the surface. PM-IRRAS experiments demonstrated that the hydration, orientation and conformation of head groups of the DMPC bilayer strongly depend on the potential, and the rearrangement of polar head groups affects packing and tilt angle of the hydrocarbon chains.

P40 **Temperature Dependence of Hydrophobic Interactions: A Monte Carlo Study**

Maria Sabaye Moghaddam, Seishi Shimizu, Hue Sun Chan (*University of Toronto*)

Hydrophobic interactions have long been hypothesized to be one of the dominant driving forces in various biomolecular processes such as protein folding. Studies involving transfer of small nonpolar solutes in water as a model system to study the hydrophobic effect on protein denaturation have flourished over the last decade. Most of the studies involving solvation of small nonpolar solutes have considered one and two methane-like solutes hydration. Two-solute hydrophobic studies, however, do not offer a complete picture of the hydrophobic interactions as they can be quite different from many body or bulk interactions.

In this work, temperature-dependent properties of hydrophobic interactions are studied by simulating the potential of mean force (PMF) amongst three methane-like solutes in TIP4P model water. Heat capacity changes upon association of three solutes are estimated to be mostly large and positive with a non-monotonic dependence on the inter-solute separation. This finding is not in agreement with solvent accessible surface area (SASA) predictions.

P41 **Gene Therapy at the Single Molecule Level: Structure and Dynamics of DNA – Cationic Lipid Complexes in Solution**

David T. Cramb (*Department of Chemistry*)

Cationic lipids are capable of delivering both hydrophobic and hydrophilic compounds¹ to the cell. Magnetic A/C mode atomic force microscopy (AFM) was used to study DNA adsorption onto supported cationic phospholipid (CL) bilayers in fluid (L_α) and gel (L_β) phase. Images of the DNA-free phospholipid bilayer revealed the dependence of supported planar bilayer structure and stability on the chemical nature of the headgroup and on the phase of the bilayer. Finally, it was illuminating to image DNA on DPTAP bilayers as a function of temperature. By examining the system above the $L_\alpha - L_\beta$ phase transition, images similar to those recorded for DNA-DOTAP(L_α) and DNA-DOTAP/DOPC(L_α) were observed. The conditions required to form transfectable lipoplexes have been extensively studied. However, to date, experiments have not addressed either the order of events of lipoplex formation in solution, or the maximum number of DNA molecules per vesicle, in stable single-vesicle lipoplexes. In the present study, we have also employed two-photon excitation fluorescence correlation spectroscopy (TPE-FCS) and two-photon fluorescence cross-correlation spectroscopy (TPE-XCS) to examine both fluorescence labelled DNA and cationic vesicles structure and dynamics simultaneously. The dependence of large aggregated lipoplex formation on DNA-cationic lipid charge ratio was determined, as was the maximum number of 40-basepair double stranded DNA oligonucleotides able to bind to a single vesicle.

P42 **Heterogeneity of fluorescence lifetimes and high exciton-exciton annihilation effects in individual chloroplasts**

Richard O. Cisek (a), Corinna Smith (a), Juerg Aus der Au (b), Jeff Squier (b), Virginijus Barzda (a) (a) *Department of Physics, University of Toronto; b) Department of Physics, Colorado School of Mines*

Multi-photon excitation, time-resolved fluorescence microscopy was used to investigate the heterogeneity of chloroplasts. We measured fluorescence lifetimes of individual chloroplasts by time correlated single photon counting. We observe heterogeneity between chloroplasts in a single cell, as well as inside chloroplasts, most probably, due to different fluorescence decays of different grana. Multi-exponential fits to fluorescence decays revealed lifetimes of less than 100ps, which is characteristic to exciton-exciton annihilation. This effect is investigated by varying laser intensities and repetition rates. Most studies of time resolved fluorescence of chloroplasts have been conducted in macroscopic measurements without the consideration of sample heterogeneity. The heterogeneity present in biological samples complicates the interpretation obtained from macroscopic fluorescence lifetime experiments. This investigation allows us to resolve the structural/functional units in thylakoid membranes, chloroplasts and other heterogeneous photosynthetic systems in vivo, and gives the opportunity to investigate the excited state dynamics of these individual units.

P43 **Structure and Thermochemical Properties of the Proton-Bound Clusters of Glycine; Formation of Peptide Bond by Ion-Molecule Mechanism in the Gas Phase.**

Serguei Raspopov, Terry McMahon (*York University*)

A new version of the Pulsed High Pressure Mass Spectrometry (PHPMS) method was developed which allows direct measurement of the thermochemical properties for non-volatile' compounds, such as aminoacids. Binding enthalpies and entropy changes were determined for proton-bound clusters of glycine. The results are in good agreement with both theoretical (DFT) calculations of this work and previous BIRD experiments. Data indicate that a number of isomers of the proton-bound dimer of glycine co-exist in the temperature range explored (360-460 K). Several new structures were found by DFT calculations, one of which represents a new energy minimum with binding energy of 122 kJ/mol. The lowest energy salt-bridged isomer is located about 7 kJ/mol higher. Another interesting and conceptually different zwitterionic isomer has a binding enthalpy of 100 kJ/mol. It is a symmetrical structure that can be described as two glycine zwitterions linked by a proton between C-termina. Experimental results also suggest that formation of peptide bond between protonated and neutral glycine residues occurs through an ion-molecule reaction, which was not observed before under thermal conditions. Kinetic data for such reaction were obtained using HPMS approach. Reaction pathway and transition states involved were also investigated by theoretical methods.

P44 **Towards Coherent Control of Light-Driven Biological Processes**

Andrea M. Nagy, Valentyn I. Prokhorenko, Steven R. Lamb, R. J. Dwayne Miller
(*Departments of Chemistry and Physics, University of Toronto*)

A directed method of laser pulse shaping is described which has been used to exploit molecular interferences between competing product channel pathways. Experiments carried out on model compounds (rhodamines in solvent) are presented, whereby the fluorescence signal is controlled by manipulating the evolution of the excited state population using tailored light pulses. Ultrafast pulses with 70nm bandwidth and compressible to < 10 fs are generated using a non-colinear optical parametric amplifier pumped with a regeneratively amplified Ti:Sapphire oscillator. Pulse shaping is carried out using an acousto-optic programmable dispersive filter, allowing for independent control of the phase and amplitude of the Fourier components of the pulse. A feedback-controlled evolutionary algorithm is used to find the optimal pulse shape for a target experimental output, namely the population of the excited electronic state. These specifically tailored excitation pulses are characterized using a transient grating Frequency Resolved Optical Gating setup. The possibility of controlling a biological process such as the photoisomerization of retinal in the protein environment (bacteriorhodopsin) will be further presented.

P45 **Femtosecond Laser Ablation of Biological Tissue: Potential medical applications and cellular mapping**

B Girard, D. Yu, B. Sukhu, C.M. Clokie, B.C. Wilson, and R.J. Dwayne Miller
(*Department of Biomedical Physics, Princess Margaret Hospital*)

One of the most important new areas for chemistry will be the development of new sub-micron spectroscopic imaging methods with near single molecule sensitivities. One of the approaches will be to use nonlinear spectroscopic methods in which femtosecond lasers are used to increase the peak power, to increase the nonlinear interaction and sensitivity, while minimizing the average power. In this regard, the effects of short laser pulses on the viability of probing single cells need to be determined. At the opposite extreme of peak power interactions, another approach is to use the enormous peak powers possible with femtosecond lasers to highly localize the photon absorption in 3 dimensions with high enough powers to ablate intracellular material for diagnostics. This approach needs to minimize any collateral damage to adjacent cellular components to enable 3-d mapping of the cellular chemistry. This latter approach has tremendous application in medicine. The application in medicine has been hampered by excessive heating of the surrounding cells leading to large necrotized zones of cell death that blocks healing. This study has explicitly used femtosecond laser ablation methods to examine the possibility of osseous surgery using 100 femtosecond pulses centered at 775nm. Key parameters related to the ablation rate, plasma shielding effects, and maximal repetition rate between pulses for fastest removal of biological tissues were examined along with ultrastructural analysis and in vitro tissue staining methods. We have been able to ablate single cells in biotissues while leaving adjacent cells intact and viable as determined by assays for cell function that specifically trace heat sensitive proteins. The results are extremely promising both for medical applications and as new methodologies for intracellular mapping of the cell.

P46 **Reduced-rotamer representations for protein structure modelling with applications to collagen fibrillogenesis**

Darren Anderson, Jan Rainey, and M.C. Goh (*Department of Chemistry, University of Toronto*)

Collagen is the most prevalent protein in mammals and in vitro self-assembles into a variety of fibrillar constructs, depending on solution conditions. A thorough understanding of the assembly process is still lacking, in part due to a lack of three-dimensional structure of the rather large monomer (3000 amino acids). By using a statistically-based reduced-rotamer representation, we have created a 3-D collagen model, and examined the location of charges as a function of pH. With this insight, we re-examine AFM results on collagen assembly mechanisms and intermediates as a function of solution conditions.