

Welcome!

In this, our fourth 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 our many sponsors. Within the University of Toronto, we gratefully acknowledge the support of the Faculty of Arts and Science, the Department of Chemistry, the Leslie Dan Faculty of Pharmacy, the Chemistry Club, and the Centre for Studies in Molecular Imaging. We also wish to express our gratitude to our corporate sponsors, including Axela Biosensors, Gamble Technologies, MDS Sciex, and Systems for Research.

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 in April, 2006. See you next year!

The Organizing Committee

Gabriel Hanna (Chair)	David McMillen (Chair)
Darren Anderson	Cecile Fradin
Gwynn Curran-Sills	Cynthia Goh
Jordan Dinglasan	Ray Kapral
Tieneke Dykstra	Dwayne Miller
Alioska Escorcía	Anja Nohe
Catherine Greenhalgh	Jeremy Schofield
Robbie Grunwald	Gilbert Walker
Aaron Kelly	
Richard Kil	
Peter Ko	
Andrea Nagy	
Jan Rainey	
Mayrose Salvador	
Nicolas Taulier	



CHEMICAL BIOPHYSICS SYMPOSIUM 2005

Department of Chemistry

University of Toronto

April 8-10, 2005

Friday, April 8

- 2:00–3:20pm Registration Koffler Centre Lobby
Door prizes provided by Houghton Mifflin Publishing
- 3:25–3:30 Introduction: David McMillen Koffler Auditorium
- Session I Chair: Gabriel Hanna Koffler Auditorium
- 3:30–4:15 a) **Leon Glass** (McGill University)
Dynamics in Genetic Networks
- 4:15–4:35 b) **Philip A. Gottlieb** (State University of New York at Buffalo)
Bilayer Dependent Inhibition of Mechanosensitive Channels by the Peptide GsMTx4
- 4:35–4:55 c) **Vali Raicu** (University of Wisconsin-Milwaukee)
Fluorescence Resonance Energy Transfer: A test-bench for fundamental laws of nature in vivo
- 4:55–5:15 d) **Vahid Shahrezaei** (Simon Fraser University)
A Modeling Study of Neurotransmitter Release: Calcium-sensor Binding Kinetics, Number of Binding Sites and Geometry of the Active Zone
- 5:15–6:00 Panel/Audience Discussion/Debate: Topic: What's the most important problem in Chemical Biophysics?
Moderator/Rabble-rouser: Regis Pomes

Advocates: **Dwayne Miller** (University of Toronto)
Leon Glass (McGill University)
Gilbert Walker (University of Toronto)
- 6:00–7:30 Buffet dinner Davenport Atrium
Coffee/beverages sponsored by Systems for Research

Friday, April 8

<u>Session II</u>	Chair: Al-Amin Dhirani	Koffler Auditorium
7:30–8:15pm	a) Paul McEuen (Cornell University) <i>Electronics, Mechanics, and Sensing with Carbon Nanotubes</i>	
8:15–8:35	b) David Cramb (University of Calgary) <i>Those Blinking Quantum Dots! A Fluorescence Correlation Spectroscopy Study</i>	
8:35–8:55	c) Rastislav Levicky (Columbia University) <i>DNA Monolayers: Properties and Applications</i>	
9:00–??	<u>Informal Discussions</u> Live music by Erlenmeyer and the Flasks.	Faculty Club Pub

Saturday, April 9

<u>Session III</u>	Chair: Jeremy Schofield	Koffler Auditorium
9:00–9:45am	a) Sharon Hammes-Schiffer (Pennsylvania State University) <i>Hydrogen Tunneling and Protein Motion in Enzyme Catalysis</i>	
9:45–10:15	b) Sergey Krylov (York University) <i>A "Swiss Army Knife" Method for Selection, Characterization, and Analytical Utilization of Aptamers</i>	
10:15–10:45	c) Yaakov Levy (Center for Theoretical Biological Physics) <i>Folding for Binding or Vice Versa: New Lessons From Minimalist Models</i>	
10:45–11:00	<u>Coffee break</u> <i>Sponsored by Gamble Technologies</i>	
<u>Session IV</u>	Chair: Anja Nohe	Koffler Auditorium
11:00–11:45am	a) James Wells (University of Toronto) <i>Oligomers, Co-operativity and Efficacy of G Protein-coupled Receptors</i>	
11:45–12:05pm	b) George Hess (Cornell University) <i>Transient Kinetic Investigations of the Mechanism of a Neurotransmitter Receptor Linked to Epilepsy</i>	
12:05–12:25	c) Supratim Sengupta (McMaster University) <i>Modeling the Evolution of the Genetic Code</i>	

Saturday, April 9

12:30–2:00pm Buffet lunch Davenport Atrium
Coffee/beverages sponsored by John Wiley & Sons, Inc.

Session V Chair: Tienieke Dykstra Koffler Auditorium

2:00–2:45pm a) **Christopher Dobson** (Cambridge University)
Protein Misfolding and its Links with Human Disease

2:45–3:05 b) **Richard Cisek** (University of Toronto at Mississauga)
Structural Investigation of Stomata and Proximity Chloroplasts with Harmonic Generation Microscopy

3:05–3:25 c) **Stefan Wallin** (University of Toronto)
Exploring the Thermodynamics of Peptide Folding and Association with a 3-letter Continuum Model

3:25–3:45 d) **Jan Rainey** (Protein Engineering Network of Centres of Excellence)
A Systematic Consideration of NMR Methods for Membrane Protein Structural Studies

Poster Session Davenport Atrium

4:00–5:30 **Even-numbered posters**

5:30–7:00 **Odd-numbered posters**
Wine and cheese sponsored by ChemClub, University of Toronto

7:00–?? Symposium Banquet ForestView Restaurant
Coffee/beverages sponsored by Axela Biosensors

Directions to the restaurant will be provided

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

Sunday, April 10

Refreshments sponsored by the Centre for Studies in Molecular Imaging

Session VI

Chair: Nicolas Taulier

Koffler Auditorium

9:30–10:15am

a) **David Andrews** (McMaster University)
The Structure of E. coli Signal Recognition Particle

10:15–10:35

b) **Lee Wilson** (University of Saskatchewan)
Interaction of Halothane With Supramolecular Compounds

10:35–11:00

Coffee break
Sponsored by MDS Sciex

11:00–11:20

b) **Andy Sinkovits** (University of Guelph)
Engineering the Catalytic Residues of Pepsin: Elucidating the Structural and Functional Role of Asp32 and Asp215

11:20–12:05pm

c) **Nadrian Seeman** (New York University)
DNA: Not Merely the Secret of Life

12:05–12:10

Closing Remarks

12:30

Lunch at a local restaurant (pay your own way)

Oral Presentation Abstracts

Ia Dynamics in Genetic Networks

Leon Glass (*Centre for Nonlinear Dynamics, McGill University*)

Genetic activity is partially regulated by a complicated network of proteins called transcription factors. I will describe a mathematical framework that can be used to relate the structure and dynamics of these genetic networks. The networks are represented by differential equations with switchlike nonlinearities. These equations are represented schematically using a directed graph on an hypercube. There are many advantages to these equations. Because of the discrete representation of the continuous dynamics, the numbers of different networks with N model genes can be counted and classified. The methods are helpful in identifying networks that have certain types of dynamic behaviors such as stable fixed points, stable cycles, and chaotic dynamics. These methods can be used to help design in vitro genetic networks that show oscillation and multistability. They can also be used to determine gene network structure based on the patterns of activation of genes (1). Finally, the framework offers novel ways to study the evolution of rhythmic patterns in model equations and also in electronic circuits that simulate the differential equations (2).

(1) T. J. Perkins, M. T. Hallett, L. Glass. Inferring models of gene expression dynamics. *Journal of Theoretical Biology* **230**, 289-299 (2004).

(2) J. P. Mason, P. S. Linsay, J. J. Collins, L. Glass. Evolving complex dynamics in electronic models of genetic networks. *Chaos* **14**, 707-715 (2004).

Ib **Bilayer Dependent Inhibition of Mechanosensitive Channels by the Peptide GsMTx4**

Philip A. Gottlieb, Thomas M. Suchyna, Sonya E. Tappe, Olaf S. Anderson, Roger E. Koeppe II, Frederick Sachs (*State University of New York at Buffalo and Cornell University Medical College*)

The peptide GsMTx4 is an inhibitor of mechanosensitive ion channels (MSCs). By NMR, we showed that it is folded into an inhibitory cysteine knot (ICK) structure. The peptide is an amphiphile, having both a hydrophobic and charged surface and acts as a gating modifier of MSCs. Using intrinsic tryptophan fluorescence, we showed that the peptide partitions into LUVs with a free energy of ~ -8 kcal/mol. To test for direct peptide-channel interactions, we synthesized the mirror-imaged GsMTx4 molecule to prevent stereospecific interactions. The enantiomeric peptide retained general physical properties found for the L-form peptide but has a reversed CD spectrum. The D-form peptide at 500 nM was as active as the L-form. We also observed that the presence of the peptide caused a reduction of inward current of about 10% (cations), but not outward currents whose ions are approaching from the opposite side of the membrane. This is consistent with GsMTx4, having a charge of +5, being within a Debye length of the channel pore. The peptide appears to mediate its effect at the membrane-channel interface. We have confirmed these membrane effects by measuring the activity of the gramicidin (gA) channel in the presence of GsMTx4. We found that GsMTx4 increases the channel activity of gA and reduces the single-channel current. We propose that GsMTx4 acts as a gating modifier on MSCs and gramicidin channels in a manner that does not conform to the traditional lock and key model. Rather, GsMTx4 alters the channel kinetics by perturbing the lipid packing adjacent to the channel.

Ic **Fluorescence Resonance Energy Transfer – a test-bench for fundamental laws of nature in vivo**

Vali Raicu (a), David B. Jansma (b), James D. Friesen (b) ((a) *University of Wisconsin-Milwaukee*, (b) *University of Toronto*)

Fluorescence Resonance Energy Transfer (FRET) – a process of nonradiative energy transfer from an optically excited molecule (donor, D) to an unexcited nearby molecule (acceptor, A) – is a powerful tool in quantitative studies of protein-protein interactions in living cells. FRET can be quantified, e.g., by measuring an increase in the donor fluorescence after inactivating the acceptor through photobleaching. In spite of its sheer simplicity, this method also introduces donor bleaching, which often complicates the interpretation of data. Correction methods are complicated by the fact that D photobleaching depends on whether a D molecule is free or coupled to an A molecule. In this communication we show that, instead of being a nuisance, donor bleaching actually can be harnessed to provide invaluable information about a population of interacting proteins in vivo. We present data on proteins tagged with fluorescent molecules, which indicate that donor and acceptor bleaching kinetics couple to the kinetics of the protein interaction, and to the diffusion of the proteins of interest in the cell. Under appropriately chosen conditions, we are able to model the bleaching kinetics of D and A (both interacting and noninteracting) and determine the stoichiometry of the protein interaction. We provide a method for imaging protein complexes in living cells (presented in more detail in a poster), which opens a way for testing the law of mass action in vivo.

Id **A modeling study of neurotransmitter release: Calcium-sensor binding kinetics, number of binding sites and geometry of the active zone**

Vahid Shahrezaei, Kerry Delaney (*Simon Fraser University*)

The brief time course of the calcium (Ca^{2+}) channel opening combined with the molecular-level colocalization of Ca^{2+} channels and synaptic vesicles in presynaptic terminals predict sub-millisecond calcium concentration ($[\text{Ca}^{2+}]$) transients of $\sim 100\mu\text{M}$ in the immediate vicinity of the vesicle. This $[\text{Ca}^{2+}]$ is much higher than some of the recent estimates for the equilibrium dissociation constant of the Ca^{2+} sensor(s) that control neurotransmitter release suggesting release should be close to saturation, yet it is well known that release is highly sensitive to changes in Ca^{2+} influx. We show that due to the brevity of the Ca^{2+} influx the binding kinetics of the Ca^{2+} -sensor rather than its equilibrium affinity determine receptor occupancy. For physiologically relevant Ca^{2+} currents and forward Ca^{2+} binding rates the effective affinity of the Ca^{2+} -sensor can be several-fold lower than the equilibrium affinity. Using simple models, we show redundant copies of the binding sites increase effective affinity of the Ca^{2+} sensor for release. Our results predict that different levels of expression of Ca^{2+} binding sites could account for apparent differences in Ca^{2+} sensor affinities between synapses. Using Monte Carlo simulations of Ca^{2+} dynamics with nanometer resolution we demonstrate that these kinetic constraints combined with vesicles acting as diffusion barriers prevent saturation of the Ca^{2+} -sensor(s) for neurotransmitter release. We further show the random positioning of the Ca^{2+} -sensor molecules around the vesicle can result in the emergence of two distinct populations of the vesicles with low and high release probability. These considerations allow experimental evidence for the Ca^{2+} channel-vesicle colocalization to be reconciled with a high equilibrium affinity for the Ca^{2+} -sensor of the release machinery.

IIa **Electronics, Mechanics, and Sensing with Carbon Nanotubes**

Paul L. McEuen (*Laboratory of Atomic and Solid State Physics, Cornell University*)

Carbon nanotubes—nanometer diameter cylinders made from rolling up single graphene sheets—are a remarkable new material for exploring the nanoscale world. This talk will review measurements by our group examining the electronic, mechanical, and chemical/biological sensing properties of nanotube devices. First, we have created nanotube field-effect transistors with significantly better intrinsic properties than Si MOSFETs. In addition, we have made (and listened to) the world's smallest guitar string by suspending nanotubes over micron-long trenches. Finally, we have explored the operation of nanotube transistors in aqueous environments for applications in sensing membranes and molecules.

IIb **Those Blinking Quantum Dots! A Fluorescence Correlation Spectroscopy Study**

Romey Heuff and David Cramb (*Department of Chemistry, University of Calgary*)

Nanocrystal semiconductor quantum dots have been touted as a new fluorescence labeling paradigm for biophysical studies. Their brightness, photostability and sharp spectral features give them great potential for multicolor imaging and correlation spectroscopy applications. One possible limitation is the propensity of the quantum dot to enter into a dark state. This phenomenon is also known as blinking. We have undertaken a fluorescence correlation spectroscopy study to examine blinking behavior of quantum dots functionalized with streptavidin and with biotin. We find that blinking is measurable using FCS and that one can also suppress this behavior.

IIc DNA Monolayers: Properties and Applications

Rastislav Levicky, Mariafrancis A. Gaspar, Patrick Johnson, George Patounakis, Gang Shen, Ken Shepard (*Departments of Chemical and Electrical Engineering, Columbia University*)

Heterogeneous hybridization, in which nucleic acid strands tethered to a solid support bind complementary nucleic acid molecules from solution, underpins DNA microarray and biosensor technologies. We investigate DNA monolayers prepared on metal and on dielectric supports, with applications to electrochemical and fluorescence-based diagnostics, respectively. Chains ranging in size from oligonucleotides to gene-sized polymers have been site-specifically attached without detectable side reactions in an end-tethered, 'polymer brush' geometry. On metal supports, single-thiol or alternately polythiol-mediated anchoring is used to provide highly permanent immobilization of the nucleic acid. This contribution will emphasize interfacial capacitance of end-tethered films of DNA chains as investigated using electrochemical impedance spectroscopy. The observed trends with ionic strength and strand surface coverage agree with physical expectations for a polyelectrolyte brush. X-ray photoelectron spectroscopy and dynamic light scattering serve as auxiliary characterization methods. More common to bioscience applications are fluorescence-based diagnostics. In this context, we are developing biochips with integrated detection and data processing circuitry. These devices seek to provide capability of macroscopic instrumentation but in a conventional CMOS chip package.

IIIa Hydrogen Tunneling and Protein Motion in Enzyme Catalysis

Sharon Hammes-Schiffer (*Department of Chemistry, Pennsylvania State University*)

Theoretical studies of proton, hydride, and proton-coupled electron transfer reactions in enzymes will be presented. We have developed a theoretical formulation for proton-coupled electron transfer reactions. The quantum mechanical effects of the active electrons, transferring proton, and donor-acceptor mode are included in this formulation, and analytical nonadiabatic rate expressions have been derived in various limits. The application of this approach to proton-coupled electron transfer in the enzyme lipoxygenase will be discussed. The experimentally measured deuterium kinetic isotope effect of 80 at room temperature is found to arise from the small overlap of the reactant and product proton vibrational wavefunctions. Our calculations illustrate that the proton donor-acceptor vibrational motion plays a vital role in the proton-coupled electron transfer reaction. We have also developed a hybrid quantum/classical molecular dynamics approach that includes electronic and nuclear quantum effects, as well as the motion of the entire solvated enzyme. The application of this approach to hydride transfer in the enzyme dihydrofolate reductase will be discussed. An analysis of the simulations leads to the identification and characterization of a network of coupled motions that extends throughout the enzyme and represents conformational changes that facilitate the charge transfer process. Mutations distal to the active site are shown to significantly impact the catalytic rate by altering the conformational motions of the entire enzyme and thereby changing the probability of sampling conformations conducive to the catalyzed reaction.

IIIb A "Swiss Army Knife" Method for Selection, Characterization, and Analytical Utilization of Aptamers

Sergey N. Krylov (*York University*)

Aptamers are DNA (or RNA) ligands selected from large libraries of random DNA sequences and capable of binding different classes of targets with high affinity and selectivity. Aptamers are often viewed as artificial antibodies and promise to revolutionize many areas of life and medical sciences [1]. Selection, kinetic characterization, and analytical utilization of aptamers conventionally requires a number of different methods including electrophoresis, filter-binding assay, surface plasmon resonance, stopped-flow spectroscopy, calorimetry, and immunoassay-like heterogeneous analyses. Here we introduce a unique method that can facilitate selection, full kinetic characterization, and analytical utilization of aptamers using a single concept, a single instrument, and a single mathematical apparatus. The method is termed non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) and dubbed a 'diagnostic Swiss Army Knife' [2]. The concept of the method will be explained and a number of its proven applications will be presented [3-12].

References

- [1] M. Rajendrani, *Anal. Chem.* 2005, 77, 115A-118A.
- [2] D. Payne, *The Scientist* 2004, 18(22), 36.
- [3] M. Berezovski, A. Drabovich, S.M. Krylova, M. Musheev, V. Okhonin, A. Petrov, S.N. Krylov, *J. Am. Chem. Soc.* 2005, 127, 3165-3171.
- [4] S.M. Krylova, M. Musheev, R. Natiu, Y. Li, G. Lee, *FEBS Lett.* 2005, 579, 1371-1375.
- [5] M. Berezovski, S.N. Krylov, *Anal. Chem.* 2005, 77, 1526-1529.
- [6] M. Berezovski, S.N. Krylov, *Anal. Chem.* 2004, 76, 7114-7117.
- [7] V. Okhonin, M. Berezovski, S.N. Krylov, *J. Am. Chem. Soc.* 2004, 126, 7166-7167.
- [8] V. Okhonin, S.M. Krylova, S.N. Krylov, *Anal. Chem.* 2004, 76, 1507-1512.
- [9] M. Berezovski, S.N. Krylov, *J. Am. Chem. Soc.* 2003, 125, 13451-13454.
- [10] S.N. Krylov, M. Berezovski, *Analyst* 2003, 128, 571-575.
- [11] M. Berezovski, R. Nutiu, Y. Li, S.N. Krylov, *Anal. Chem.* 2003, 75, 1382-1386.
- [12] M. Berezovski, S.N. Krylov, *J. Am. Chem. Soc.* 2002, 124, 13674-13675.

IIIc **Folding for binding or vice versa: New lessons from minimalist models**

Yaakov Levy, Jose N. Onuchic, Peter G. Wolynes (*Center for Theoretical Biological Physics*)

Transient interactions between both proteins and nucleic acids are ubiquitous and fundamental to many subcellular processes. We have recently shown that binding mechanisms are robust and owing to the minimal frustration principle, just as for protein folding, are governed primarily by the protein's native topology, which is characterized by the network of non-covalent residue-residue interactions (1, 2). The native topology based landscape model, which corresponds to a perfectly funneled energy landscape, reproduces many of the grosser and finer structural and kinetic aspects of various binding mechanisms found in the laboratory (3). Not only are our computational results consistent with experiments, they also demonstrate that protein plasticity, as envisioned by the fly-casting mechanism, is more fundamental in protein recognition than traditionally imagined. In protein-DNA recognition (4), both fly-casting effect and electrostatic forces contribute to an efficient binding. An asymmetric binding mechanism is often observed for the formation of the symmetric homodimers where one monomer is more structured at the binding transition state. The protein topology was successfully implemented to predict symmetric complexes that are formed via domain-swapping (5). Finally, we propose resolutions of the longstanding experimental puzzles on the dimerization of HIV-1 protease (6) and the association kinetics of Rop dimer (7).

1. Levy, Y., Wolynes, P. G. & Onuchic, J. N. (2004) Proc Natl Acad Sci USA 101, 511-516.
2. Levy, Y., Papoian, G. A., Onuchic, J. & Wolynes, P. G. (2004) Isr J Chem 44, 281-297.
3. Levy, Y., Cho, S. S., Onuchic, J. N. & Wolynes, P. G. (2005) J Mol Biol 346, 1121-1145.
4. Levy, Y. & Onuchic, J. N. (2004) Proc Natl Acad Sci USA 101, 3325-3326.
5. Yang, S., Cho, S., Levy, Y., Cheung, M. S., Levine, H., Wolynes, P. G. & Onuchic, J. N. (2004) Proc Natl Acad Sci USA 101, 12786-12791.
6. Levy, Y., Caffisch, A., Onuchic, J. N. & Wolynes, P. G. (2004) J Mol Biol 340, 67-79.
7. Levy, Y., Cho, S. S., Shen, T., Onuchic, J. N. & Wolynes, P. G. (2005) Proc Natl Acad Sci USA 102, 2373-2378.

IVa **Oligomers, Co-operativity and Efficacy of G Protein-coupled Receptors**

James W. Wells (*Leslie Dan Faculty of Pharmacy, University of Toronto.*)

G protein-coupled receptors undergo an enigmatic process that underlies signalling and a clutch of related phenomena such as efficacy, constitutive activity and a characteristic heterogeneity revealed in the binding of agonists. It appears to involve the spontaneous interconversion of the receptor between at least two states—one active and one inactive—that remain undefined in molecular terms. The common view has long been that signalling occurs via a transient 1:1 complex between the receptor and the G protein (i.e., $R + G \rightleftharpoons RG$); thus, heterogeneity was thought to be induced by the G protein in a population of mutually independent and otherwise identical receptors. That view is problematic, however, in that it fails to account for the binding properties of receptors and G proteins in a quantitative and mechanistically consistent manner; moreover, it is at odds with evidence that G protein-coupled receptors exist as oligomers and that the interaction with the G protein is not spontaneously reversible. An alternative view holds that signalling and related properties, such as efficacy and heterogeneity of binding, derive from co-operative interactions within an hetero-oligomeric array that comprises multiple equivalents of receptor and G protein. Co-operativity has been identified in the binding of ligands to the m2 muscarinic cholinergic receptor in various preparations, including detergent-solubilised preparations. Mechanistic models that can account for such effects require at least four interacting sites to describe the data. Homo-oligomers of the receptor have been identified by their electrophoretic mobility, co-immunoprecipitation and fluorescence resonance energy transfer; estimates of their size point to tetramers or larger aggregates.

IVb **Transient Kinetic Investigations of the Mechanism of a Neurotransmitter Receptor Linked to Epilepsy**

George P. Hess, Latha Ramakrishnan, Yang Cui (*Cornell University*)

A laser-pulse photolysis technique for investigating membrane-bound proteins with microseconds resolution (Hess & Grewer 1998) was used to characterize the dysfunction of a mutated GABA(A) receptor linked to epilepsy (Baulac et al 2001). The mutated receptor was expressed in HEK293 cells. Cells in the whole-cell current-recording mode were equilibrated with a photolabile precursor of GABA (N-alpha-carboxy-2-nitrobenzyl GABA) (Gee et al 1994)). The reaction was initiated by irradiation for 10 microseconds, and rate constants for channel opening (k_{op}) and closing (k_{cl}) and the binding affinity of GABA determined. The ratio of k_{op}/k_{cl} , the channel-opening equilibrium constant, was much lower in the mutated receptor than in the wild-type receptor, accounting for the reported inefficiency of the mutated receptor (Ramakrishnan & Hess 2004). This information was used to identify ligands that change the channel-opening equilibrium. Picrotoxin, an inhibitor of the receptor (De Deyn et al 1990), was identified as such a ligand and used to isolate combinatorially synthesized RNA ligands that bind with higher affinity to the picrotoxin-binding site on the open- rather than closed-channel receptor form (Cui et al 2004). The RNA ligands alleviated picrotoxin inhibition and favorably affected the dysfunctional mutated receptor. Transient kinetic investigations had led to an understanding of the mechanism of dysfunction of the mutated receptor and to an approach to alleviate the dysfunction.

IVc **Modeling the Evolution of the Genetic Code**

Supratim Sengupta, Paul Higgs (*McMaster University*)

Many modified genetic codes are found in specific genomes in which one or more codons have been reassigned to a different amino acid from that in the canonical code. We present a model that unifies four possible mechanisms for reassignment, based on the observation that reassignment involves a gain and a loss. The loss could be the deletion or loss of function of a tRNA or release factor. The gain could be the gain of a new type of tRNA for the reassigned codon, or the gain of function of an existing tRNA due to a mutation or a base modification. We present simulations of the gain-loss model and demonstrate that all four mechanisms are possible. By following the order of events in our codon reassignment simulations, we are able to distinguish between the various evolutionary pathways of codon reassignment. The frequencies of the different mechanisms are influenced by selection strengths, number of codons undergoing reassignment, directional mutation pressure and the possibility of selection for reduced genome size.

Va **Protein Misfolding and its Links with Human Disease**

Christopher M. Dobson (*Department of Chemistry, University of Cambridge*)

There has been considerable progress in the last few years in understanding the underlying principles that govern the process of protein folding. We have been interested recently in the relationship of normal folding to misfolding events, particularly those associated with human disease. We have been investigating in particular the nature of the amyloidogenic conditions, that include Alzheimer's disease, type II diabetes and the spongiform encephalopathies, e.g. BSE and CJD, in which protein misfolding leads to the aggregation of proteins, often into thread-like amyloid structures. Our studies have led us to put forward ideas concerning the fundamental origins of the various diseases associated with their formation and the various strategies that can be used for their prevention and treatment. We have also speculated more generally that the need to avoid aggregation could be a significant driving force in the evolution of protein sequences and structures.

References

- C.M. Dobson, Protein Folding and Misfolding, *Nature* **426**, 884-890 (2003).
- M. Dumoulin, A.M. Last, A. Desmyter, K. Decanniere, D. Canet, G. Larsson, A. Spencer, D.B. Archer, J. Sasse, S. Muyldermans, L. Wyns, C. Redfield, A. Matagne, C.V. Robinson and C.M. Dobson, A Camelid Antibody Fragment Inhibits the Formation of Amyloid Fibrils by Human Lysozyme, *Nature* **424**, 783-788 (2003).
- C.M. Dobson, Protein Folding and Misfolding, *Nature* **426**, 884-890 (2003).
- F. Chiti, M. Stefani, N. Taddei, G. Ramponi and C.M. Dobson, Rationalisation of Mutational Effects on Protein Aggregation Rates Using Simple Physical Principles, *Nature* **424**, 805-808 (2003).
- C.M. Dobson, In the Footsteps of Alchemists, *Science* **304**, 1259-1262 (2004).
- D.M. Korzhnev, X. Salvatella, M. Vendruscolo, A.A. Di Nardo, A.R. Davidson, C.M. Dobson and L.E. Kay, Low-populated Folding Intermediates of Fyn SH3 Characterized by Relaxation Dispersion NMR, *Nature* **430**, 586-590 (2004).
- K. Lindorff-Larsen, R.B. Best, M.A. Depristo, C.M. Dobson and M. Vendruscolo, Simultaneous Determination of Protein Structure and Dynamics, *Nature* **433**, 128-132 (2005).

Vb **Structural Investigation of Stomata and Proximity Chloroplasts with Harmonic Generation Microscopy**

Richard Cisek, Nicole Prent, Catherine Greenhalgh, Arkady Major and Virginijus Barzda (*Department of Physics, University of Toronto*)

Three dimensional *in vivo* structural investigations of guard cells and mesophyll chloroplasts were performed using nonlinear femtosecond laser scanning microscopy with simultaneous detection of third harmonic generation, second harmonic generation, and multiphoton excitation fluorescence. The third harmonic was efficiently generated from chloroplasts most probably due to the multilamellar structure of grana and the high carotenoid content in light-harvesting pigment-protein complexes. Efficient second harmonic generation was observed from guard cells of the stomata, however, the origin of the signal is still under investigation. Significant second harmonic signal also originated from microcrystalline starch granules and from the chiral structure of grana in chloroplasts. In addition, three dimensional images of chloroplasts and stomata were obtained by multiphoton excitation (auto)fluorescence microscopy. These fluorescence images serve as references for comparisons with images generated from the third and second harmonics. All three microscopic signals are sensitive to the plant's physiological state. The newly developed nonlinear microscopy method with three contrast mechanisms is very useful for studying light induced structural and functional dynamics of stomata movements. In addition, this method facilitates the investigation of the role of chloroplasts in light regulated stomata movement.

Vc **Exploring the thermodynamics of peptide folding and association with a 3-letter continuum model**

Stefan Wallin, Hue Sun Chan (*University of Toronto*)

We present a novel continuum protein model with 3 different amino acid types — hydrophobic, polar, and glycine — that allows us to predict the free-energy minima of a set of designed peptides involving both α -helical and β -sheet structure. This set of designed sequences, which range from 16 to 54 amino acids, include the aggregation-prone *ccb* peptide (Dobson et al. PNAS **101**, 4435–4440, 2004), a three-helix bundle, and a three-stranded β -sheet peptide. The model has 7 atoms per amino acid; all backbone atoms are explicitly included while side-chains are represented by a single atom, a large C_β . The potential is deliberately kept simple and based mainly on hydrogen bonds and effective hydrophobic forces (no explicit water), and effective Monte Carlo methods are used to calculate the thermodynamic behavior of the peptide chains. Without resorting to parameter changes, we find that the various peptide chains make abrupt transitions from expanded conformations at high temperatures T , to compact states at low T that correspond well to the intended target structures, showing that the model is able to treat both α -helical and β -sheet structures despite its relatively simple energy function. In addition, we perform simulations of 2- and 3-chain systems in periodic boundary conditions of the *ccb* peptide and an “ideal” amphiphilic α -helix. Specifically, for three chains, we find the probability for the *ccb* peptide system to populate a β -sheet rich aggregated state to be significantly larger than for the amphiphilic α -helix system, which tends to form coiled-coil structures at low T .

Vd **A systematic consideration of NMR methods for membrane protein structural studies**

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

Membrane proteins are notoriously difficult to study. A membrane protein generally has three domains in three very different environments: the extracellular space, the cell membrane, and the cytosol. Intermolecular interactions between the protein and each of these environments, as well as intramolecular interactions, all contribute to the native structure of the protein. Using solid-state NMR, it is possible to study proteins directly in phospholipid bilayers. However, such studies are still not routine because of the inherent low sensitivity of the NMR signal and the low experimental resolution in the solid-state. Using solvents of low dielectric, ‘piece-wise solution of individual segments of membrane proteins have been shown to provide structures often similar to the corresponding segment of the crystal structure of the entire protein. Detergent micelles provide a potentially more physiological environment for such solution-state studies. We have studied transmembrane peptides in such ‘membrane mimetic solvents and in micelles which demonstrate that the NMR derived structure is not necessarily independent of the solution conditions. In the course of these studies we have re-examined some of the typical assumptions made in the literature, such as the suitability of aqueous state standards for studies at a low dielectric. As a stepping stone towards determination of the relationship between solution and bilayer derived structures, we present an optimization of conditions to produce oriented bilayer samples and the development of NMR methods to allow use of data from sample rotation relative to the static magnetic field of the spectrometer.

VIa **The Structure of *E. coli* Signal Recognition Particle**

David W. Andrews (*Biochemistry and Biomedical Sciences, McMaster University*)

We describe a structure for the complete Signal Recognition Particle (SRP) from *E. coli*. SRP is a complex of protein(s) and RNA found in all cell types that facilitates the movement of proteins across or into membranes. Although the subject of several previous structural studies no complete structure of bacterial SRP has emerged that is consistent with the biochemistry of the particle. Furthermore, these structures have not provided significant new biological insight into how SRP actually functions. By using a combination of structural techniques and modeling we propose a new model for how SRP interacts with the ribosome at the exit site for new proteins being made by the ribosome.

To reconstruct a 3-dimensional structure of SRP including the full protein and RNA we used cryo-scanning transmission electron microscopy to collect 3,236 images of *E. coli* SRP particles. The phosphorus-containing RNA was mapped within the SRP reconstruction by electron spectroscopic imaging. When this data were used to orient crystallographic data for Ffh from *Thermus aquaticus* and *Sulfolobus solfataricus* within the *E. coli* SRP reconstruction, the resulting model differed from the structures of SRP from these organisms but all three structures could be rationalized by movement through an interdomain linker. Fluorescence resonance energy transfer experiments determined interdomain distances consistent with our model of SRP.

Most importantly, docking our model onto the structure of the bacterial ribosome suggests a model for signal recognition involving interdomain movement of Ffh into and out of the nascent chain exit site and suggests how SRP could interact and/or compete with the ribosome-bound chaperone, Trigger Factor, for a nascent chain during translation.

VIb Interaction of Halothane With Supramolecular Compounds

Lee D. Wilson, Ronald E. Verrall, Xiaoguang Wen (*Department of Chemistry, University of Saskatchewan*)

NMR and high precision densimetry were utilized to investigate the nature of the interaction between anesthetics and various cyclodextrins and polymer compounds in aqueous solution at 293 K. Solutions of halothane in the presence and absence of supramolecular compounds were prepared in flame sealed ampoules. NMR chemical shift and high precision density measurements were obtained for halothane in the presence and absence of supramolecular compounds. ROESY NMR spectra were recorded for halothane in the presence of cyclodextrins and polymers at various mixing ratios and mixing times. The complexation induced shifts (CIS) and apparent molal volumes (AMV) were analyzed to determine the stoichiometry, binding constants, and changes in hydration of the anesthetic molecules. CIS values, ROESY data, and AMV results show positive correlations and indicate that there are significant interactions between the cyclodextrin macrocycle and halothane, according to the hydrophobic effect. Similar observations are made for halothane-polymer systems, however; there are significant interactions between lipophilic and hydrophilic regions of the polymer. Calculations reveal that several water molecules are involved in the hydration change upon complex formation. These results indicate that inclusion binding is operative and that binding between the inhalation anesthetic and periphery of the cyclodextrin macrocycle are also important. In the case of polymer systems, substantive interactions are observed in the hydrophobic and interfacial domains of the polymer structure. These results contribute to the general mechanism of anesthetic action.

VIc **Engineering the catalytic residues of pepsin: elucidating the structural and functional role of Asp32 and Asp215**

Andy F. Sinkovits, Takuji Tanaka, Rickey Y. Yada (*University of Guelph, Department of Food Science*)

Amino acid substitutions were made in three conformations to the catalytic residues of pepsin, namely, Asp32 and Asp215. Mutants D32E, D215E, and D32E/D215E showed marked differences in various parameters as compared to wild-type pepsin (WT). All mutants showed a complete inability to autocatalytically activate in acid. Mutant D32E showed a marked decrease in activity at pH 1.5 relative to WT, suggesting a change in the normally conserved pK_a of residue 32. Mutants D215E and D32E/D215E showed no significant difference in the pH optima as compared to WT. All three mutants showed lower affinity for substrate, decreased turnover numbers, and decreased catalytic efficiency. Circular dichroism spectropolarimetry (CD) investigation revealed that the mutations did not alter the secondary structure organization of the enzymes as compared to WT. Thermal denaturation using CD showed decreased melting temperatures (T_m), decreased Gibb's free energy values (ΔG°), decreased enthalpy (ΔH°), and decreased entropy (ΔS°), suggesting a disruption of the hydrogen-bond network of the active site and offered an explanation of the decreases in catalytic efficiency. Additionally, energy minimizations showed the conformation of Glu32 to be out of plane with Asp215 in D32E. Glu215 appeared to be shielded from changes in coordinate position by Thr218 in the D215E mutant which suggested a critical supportive role for residue Thr218. The D32E/D215E mutant showed a relative shift along the plane of both catalytic residues which was inadequate to preserve the catalytic efficiency of the enzyme. D32E showed a global reduction in flexibility, whereas D215E and D32E/D215E showed more localized changes. The data suggested that the hydrogen-bond network of the active site not only provides rigidity to the catalytic apparatus, but is also integral to the ionization of the catalytic residues of pepsin. The decreased rigidity of the active site may allow the lytic water molecule to oscillate and thus decrease the catalytic efficiency of the mutants.

VIId DNA: Not Merely the Secret of Life

Nadrian C. Seeman (*Department of Chemistry, New York University*)

Structural DNA nanotechnology uses the concept of reciprocal exchange between DNA double helices or hairpins to produce branched DNA motifs, like Holliday junctions, or related structures, such as double crossover (DX), triple crossover (TX), paranemic crossover (PX) and DNA parallelogram motifs. We have been working since the early 1980's to combine DNA motifs, using sticky-ended cohesion, to produce specific structures. The key strength of sticky-ended cohesion is that it produces predictable adhesion combined with known structure. From branched junctions, we have constructed DNA stick-polyhedra, whose edges are double helices, and whose vertices are the branch points of DNA branched junctions. These include a cube, a truncated octahedron, and an irregular graph. This approach has also rendered accessible several topological targets, such as deliberately designed knots and Borromean rings. Recently, we have begun to template the topology of industrial polymers, such as nylon with DNA-like scaffolds.

Nanorobotics are key to the success of nanotechnology. We have used two DX molecules to construct a DNA nanomechanical device by linking them with a segment that can be switched between left-handed Z-DNA with right-handed B-DNA. PX DNA has been used to produce a robust sequence-dependent device that changes states by varied hybridization topology. The sequence-dependent nature of this device means that a variety of them attached to a motif can all be addressed individually. Recently, we have used this device to make a translational device. A protein-activated device that can be used to measure the ability of the protein to do work, and a bipedal walker have both been built.

A central goal of DNA nanotechnology is the self-assembly of periodic matter. We have constructed micron-sized 2-dimensional DNA arrays from DX, TX and two kinds of parallelogram motifs. We can produce specific designed patterns visible in the AFM from DX and TX molecules. We can change the patterns by changing the components, and by modification after assembly. In addition, we have generated 2D arrays from DNA parallelograms. These arrays contain cavities whose sizes can be tuned by design. Recently, we have used new motifs to produce honeycomb-shaped arrays.

The key structural challenge in the area is the extension of the 2D results obtained so far to 3D systems with a high degree of ordering. Several motifs have been produced that can produce 2D arrays in each of the three directions normal to the vectors that span the 3D space. Crystals with dimensions as large as a millimeter, ordered to 10 Å resolution (as determined by X-ray diffraction) have been produced. Ultimately, we expect to be able to produce high resolution crystals of DNA host lattices with heterologous guests, leading to well-ordered bio-macromolecular systems amenable to diffraction analysis. Other challenges are to incorporate DNA nanomechanical devices in periodic and aperiodic lattices and to use the lattices to organize nanoelectronic components, such as metallic nanoparticles or carbon nanotubes.

Poster Presentation Abstracts

Saturday, April 9, 2005

All poster presenters set up their posters after Session V (3:45pm), then be available near their poster for half of the session, as follows:

Even-numbered posters: 4:00–5:30pm

Odd-numbered posters: 5:30–7:00pm

P1 **An Atomic Force Microscope Based DNA Sequencing Technique**

Quinn Spadola, Brian Ashcroft, Shahid Qamar, Peiming Zhang, Stuart Lindsay
(*The Biodesign Institute, Arizona State University*)

We are developing an atomic force microscope (AFM) based method of sequencing DNA. We have formed a rotaxane consisting of single stranded DNA and a cyclodextrin ring bound at the 5' end to a silicon substrate. The cyclodextrin is functionalized in order for it to bind to an AFM cantilever tip. Our intention is for the tip to approach the surface and bind to the cyclodextrin. As it is lifted it will pull the cyclodextrin over the single stranded DNA. The AFM is used to detect the forces required to slip the cyclodextrin over the single stranded DNA. As the ring slides over each base we expect a different amount of force to be applied to the cantilever. The difference in size and chemical properties between the bases should allow this method to be used as a sequencing technique.

P2 Two – Photon Excitation Fluorescence Cross – Correlation Spectroscopy for Novel Ligand Binding Assays

Jody L. Swift, David T. Cramb, Tanya E.S. Dahms, Romey Heuff (*University of Calgary*)

The in vitro assessment of large libraries of potential therapeutic agents can be achieved using high throughput screening (HTS). Conventionally, fluorescence based assays are used for HTS due to low cost output and availability of commercial detection equipment. However, fluorescence based high context screening (HCS) of live cells has been limited by intrinsic fluorescence of many cellular components. Large backgrounds arising from high levels of autofluorescence from the sample result in high instances of false positive tests. Additionally, many fluorescence assays report on secondary effects of ligand receptor interaction, such as increases in intracellular Ca^{2+} levels, rather than direct ligand binding. Fluorescence cross-correlation spectroscopy (XCS) can be utilized to address the limitations of current fluorescence based high context screening, allowing for direct detection of ligand-receptor interactions. In XCS interacting species are labeled with fluorophores of different and nonoverlapping spectra. Two-photon excitation (TPE) enables the simultaneous excitation of both fluorophores using a single wavelength. Emission from the sample is spectrally separated and collected simultaneously in two separate detection channels. A cross-correlation signal is obtained only when the two distinctly labeled species are physically linked, and the uncorrelated signal (autofluorescence) will time average to zero. Thus, XCS is ideal for tracking the dynamics of dimerized (dually labeled) species amidst a sea of monomers. Biotin – Streptavidin, a simple ligand binding system has been used to validate this method for abstracting the desired pharmacological parameters. Recent developments will be described illustrating the use of TPE–XCS for monitoring binding of various ligands to a model G–protein coupled receptor (GPCR), the human mu opioid receptor (hMOR). Using this technique it is possible to simultaneously follow the kinetics of binding in addition to the changes in the structures of the bound species.

P3 The in ovo Chick Chorioallantoic Membrane as a Model of Two-Photon Excitation PDT for Age-Related Macular Degeneration

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

Two-photon excitation photodynamic therapy (TPE-PDT) is a promising treatment for people suffering from wet age-related macular degeneration (AMD), the leading cause of blindness in people over the age of 60. Wet AMD involves the outgrowth of blood vessels from the back of the eye into the retinal tissue causing structural and subsequently, visual damage. Existing treatment regimes available for AMD, such as one-photon excitation PDT, can cause further vision loss from thermal heating and out-of-focus photosensitizer activation. TPE-PDT therapy uses lower energy near-infrared radiation and has a confined focal volume (~ 102 fL). Therefore, TPE-PDT has the potential to reduce collateral damage to the sensitive retinal tissue surrounding the new vasculature. The 8-9 day chick chorioallantoic membrane (CAM) is an excellent model for AMD due to the presence of blood vessels undergoing rapid angiogenesis that are embedded in transparent membranes. The in ovo, or 'in egg', chick model has been developed in order to achieve intravascular micro-injection of the photosensitizer. Currently therapeutic parameters such as drug dose, and exposure time and intensity for TPE-PDT in the CAM are being investigated to find the optimum treatment criteria.

P4 Conformational change of calmodulin detected by thickness shear mode acoustic wave technology

Xiaomeng Wang, Emma-Louise Lyle, Priyanka Sundaram, Michael Thompson (*Department of Chemistry, University of Toronto*)

Conformational change of calmodulin (CaM) upon binding with ions or peptide was detected by thickness shear mode (TSM) acoustic wave technology. It offers us another novel methodology for immobilized protein conformation detection besides surface plasmon resonance. Biotinylated CaM was immobilized by avidin-biotin linkage onto the gold electrode surface of the sensor. Interactions between CaM and calcium ions/magnesium ions have induced resonance frequency increase and motional resistance decrease, which were reversible after buffer washing. Different degrees of frequency change generated by different ions proved different conformations of CaM upon binding events. Furthermore, binding of CaM with peptide plus calcium ions was also investigated, which showed us a more steady calcium ion-dependent CaM-peptide interaction. It has been calculated by us that the frequency increase was not related to mass loss from the surface. Therefore, we confirmed that TSM instrument could be used to detect the conformational change of CaM protein. In the near future, it will be used economically in the area of medical community and drug discovery giving responses to any protein conformational changes.

P5 Exploring Hydrogen/Deuterium Scrambling Through Mass Spectrometry of Specifically Designed Peptides

John K. Chik, David C. Schriemer (*University of Calgary, Faculty of Medicine, Department of Biochemistry and Molecular Biology*)

By following mass changes of peptide fragments, hydrogen/deuterium exchange mass spectrometry (HDX MS) shows great promise for studying protein interactions by simultaneously locating binding sites and binding induced conformational changes. Reliance on enzymatically derived fragments however imposes limits on the ability to ‘resolve’ sites of deuteration. It has been suggested that tandem MS methods could greatly improve HDX MS resolving power provided that fragmentation itself does not induce intra-fragment ‘scrambling’ of protons and deuterons. We have studied the HDX kinetics of a series of synthetic peptides paying close attention to the overall population distribution of incorporated deuteriums. Taken together, the data questions the notion that rates can be associated to specific amides. Rather, the MS observed exchange rates are a function of the total peptide and not a sum of the amides. Our data is consistent with a model in which different exchange mechanisms have specific probabilities of occurring at each backbone amide. This model does not require the rearrangement of protons/deuterons and holds out some hope for the careful use of tandem MS to further localize deuteration.

P6 Modeling Studies of TatA from the Twin-Arginine Translocase System.

Marian R. Zlomislic, Cathy S. Chan, R. J. Turner, D. Peter Tieleman (*Department of Chemistry and Department of Biological Sciences, University of Calgary*)

The twin-arginine translocase (Tat) system has the remarkable ability to transport fully folded proteins of a range of sizes across the periplasmic membrane of most bacteria. The system functions with a simple set of subunits, working together in a yet unknown mechanism. One of the three major subunits is TatA, an 89 residue protein that can be truncated to 49 N-terminal residues and still retain Tat function. It is believed that many units of TatA form the translocating channel. To understand how such a channel might function, we are using a variety of modeling methods including simulated annealing and molecular dynamics simulations to study multimeric TatA structures. In combination with principles of membrane protein structure and available experimental data, our goal is to propose and discount potential mechanisms for the translocase channel.

P7 Characterization of Quantum Dot behaviour in live mammalian cells

Ajit Thakur, Cecile Fradin (*McMaster University*)

Quantum dots (QDs) are semiconductor nanocrystals with exceptional optical properties that make them ideal candidates for use as fluorescent probes in single molecule studies. Of particular interest for biological investigations are quantum dots rendered water-soluble by the addition of an external hydrophilic layer. Yet, the fact that these inorganic nanocrystals might be toxic for living organisms remains a concern. We consequently tried to establish how living culture cells would react to the introduction of different types of water-soluble quantum dots in their cytoplasm. In this investigation, quantum dots were first characterized in solution with fluorescence correlation spectroscopy (FCS), time-lapse video microscopy (TLVM), fluorimetry and atomic force microscopy (AFM) for their fluorescence properties and size distribution. We verified that quantum dots are highly photostable and exhibit characteristic blinking. We also found that some of them had a tendency to aggregate in solution probably due to an imperfect hydrophilic layer. The quantum dots were next loaded into live mammalian cells using a non-invasive technique and their motion followed using time-lapse video microscopy. While the cells seemed unperturbed by the presence of single quantum dots, large aggregates were clearly identified as foreign bodies, as shown by their localization into acidic structures called lysosomes, and directed for destruction. Our results highlight the importance of using quantum dots coated with a proper hydrophilic layer for live cell applications.

P8 Transport Properties of Quantum-Classical Systems

Hyojoon Kim, Raymond Kapral (*Chemical Physics Theory Group, Department of Chemistry, University of Toronto*)

Correlation function expressions for calculating transport coefficients for quantum-classical systems are derived. The results are obtained by starting with quantum transport coefficient expressions and replacing the quantum time evolution with quantum-classical Liouville evolution, while retaining the full quantum equilibrium structure through the spectral density function. The method provides a variety of routes for simulating transport coefficients of mixed quantum-classical systems, composed of a quantum subsystem and a classical bath, by selecting different but equivalent time evolution schemes of any operator or the spectral density. The structure of the spectral density is examined for a single harmonic oscillator where exact analytical results can be obtained. The utility of the formulation is illustrated by considering the rate constant of an activated quantum transfer process that can be described by a many-body bath reaction coordinate.

- P9 **Exploring the biomineralization process of collagen and hydroxyapatite**
Ming Sun, Adam Drover, Erika F. Merschrod S. (*Department of Chemistry, Memorial University of Newfoundland*)

Our work has been focusing on the formation of biomineralization materials that are primarily a composite of the protein collagen and hydroxyapatite. Earlier work in our group indicated that collagen monomers and fibrils have different effects on the crystal structure of the mineral formed. How the two distinct procedures of the fibrillogenesis of collagen and the precipitation of calcium phosphate affect each other during biomineralization process in vitro on the nano-to-mesoscale are explored by AFM (atomic force microscope) in situ.

- P10 **Simulation Studies of peptide-membrane interaction**

Bruno Tomberli, Viktor Vivcharyuk, Chris G. Gray, Saul Goldman (*University of Guelph*)

A simulation protocol, developed for measuring the potential of mean force (PMF) as a function of distance between an antimicrobial peptide (e.g. lactoferricin) and the inner and outer membranes of a gram-negative bacteria is discussed. The proposed method directly measures the PMF by constraining the peptide within a slab of thickness dz at height z above the membrane and measuring the external force on the peptide. Preliminary results will be presented along with future plans using alternative methods.

- P11 **Spectroelectrochemical study of cell-to-cell fusion in living cells**

Jie Li, Grzegorz Szymanski, Jacek Lipkowski (*University of Guelph*)

Cell fusion has great potential in membrane research. Cell biologists, virologists, biochemists and biophysicists all share the interest in studying membrane fusion. Fusion always involves the merger of two membrane lipid bilayers. In spite of the very intensive experimental work in different labs, the multistep conformational changes in lipid bilayers still remain unknown. Surface Enhanced Infrared Absorption Spectroscopy, which is based on Attenuated Total Reflection, will be used to study the conformational changes of the lipid bilayers in the cell membrane during the electric field mediated cell-to-cell fusion in living cells.

P12 Adsorption of decyltrimethylammonium triflate (DeTATf), a model cationic surfactant, at the Au(111) surface

Christa L. Brosseau, Erin Sheepwash, Ian Burgess, Ewa Cholewa, Jacek Lipkowski, Sharon Roscoe (*University of Guelph*)

Electrochemical measurements including cyclic voltammetry, differential capacity and chronocoulometry have been used to characterize the adsorption behaviour of the cationic surfactant decyltrimethylammonium triflate (DeTATf) at the Au(111) surface. Cyclic voltammograms showed the presence of a major reversible adsorption / desorption peak at -0.5 V, which shifted progressively towards more negative potentials with increasing concentration. This indicated increased thermodynamic stability as the bulk concentration was increased. A minor peak was observed at 0.15 V which was indicative of a phase transition. Differential capacitance measurements also showed these two peaks, and the phase transition was observed to be eliminated at a DeTATf concentration which was much greater than the critical micelle concentration for the surfactant. Charge density curves for DeTATf reflected the adsorption behaviour nicely, with both the main adsorption and the phase transition observed as regions of increased slope. In addition, the thermodynamics of this ideally polarizable electrode have been employed to determine the Gibbs excess of adsorption.

The results from this work show that the adsorption of DeTATf is nearly identical to that observed for a previously studied zwitterionic surfactant of similar structure, and therefore the cationic surfactant studied presently appears to be coupled to the triflate counter ion and adsorbs essentially as an ion pair at the Au(111) surface.

P13 Measurement of Adhesion Energy and Young's Modulus in Thin Polymer Films Using a Novel Axi-symmetric Peel Test Geometry

Adam N. Raegen, Kari Dalnoki-Veress (*Department of Physics & Astronomy and the Brockhouse Institute for Material Research, McMaster University*)

We present a method of probing adhesion between solids, particularly in systems involving polymers. This method uses the axi-symmetric deformation of a thin spin-cast polymer membrane brought into contact with a film supported on a substrate to measure the work of adhesion between the pair. In this geometry, the contact area and constitutive relation (force versus displacement curve), are measured. This enables the determination of Young's modulus, surface energy, and the pretension of the free-standing film, which are in good agreement with accepted values.

P14 A novel approach to measurement of the adhesion strength of a single cell on a substrate

Marie-Josée Colbert, Kari Dalnoki-Veress, Cecile Fradin (*Department of Physics & Astronomy and the Brockhouse Institute for Material Research, McMaster University*)

The fundamental study of the adhesion of cells on solid surfaces is crucial to the characterization and development of materials suitable for use in biological environments (i.e. implants). We will present our work on the adhesion of a single vesicle on a substrate. A vesicle is held at the end of a micropipette mounted on a micromanipulator and put into contact with a surface. Adhesion is measured by pulling the vesicle from the substrate. Rather than using suction to infer the adhesion strength, we take advantage of the spring constant of an L-shaped micropipette to directly measure the adhesion force. The effect of surface roughness on the adhesion strength of vesicles will be discussed.

P15 Diffraction Sensing and Polyelectrolyte Multilayer Applications

Matthew M. Coulter, Richard W. Loo, M. Cynthia Goh (*Department of Chemistry, University of Toronto*)

We describe diffraction sensing of protein-protein interactions. Proteins such as immunoglobulin G can be stamped onto a glass slide via microcontact printing. When subject to a laser beam, the protein layer produces a diffraction pattern due to the periodic nature of the stamp. Correlating the intensity of the diffracted order with binding events allows kinetics of binding to be examined in the context of concentration and temperature dependence. A combinatorial strategy for biological patterning of surfaces and multilayer film synthesis is demonstrated via the construction of many polyelectrolyte multilayer films in 96-well microtitre plates. The effect of different polyelectrolyte primer layers and of varying the salt content of polyelectrolyte solutions on polyelectrolyte multilayer film formation is investigated via the construction of a library of 120 distinct poly S119/polydiallyldimethylammonium chloride (PDDA) multilayer films using the combinatorial approach.

P16 Chlorophyll-driven folding of light-harvesting proteins

Craig C. Jolley, Petra Fromme, Mike Thorpe (*Arizona State University*)

The initial step in the process of photosynthesis is carried out by light-harvesting proteins, which use non-covalently bound pigments such as (bacterio)chlorophyll and carotenoids to absorb light energy and efficiently transfer it to a photochemical trapping site. Most of these light-harvesting proteins are large, multi-subunit membrane protein complexes. Although several X-ray structures have been determined for these proteins (1-4), the folding and assembly of these complicated protein-cofactor complexes is still an unsolved problem. The graph-theoretical algorithm FIRST has been used in the past to study the folding of soluble proteins based only on their native structure, as determined by X-ray crystallography (5-7). We have modified the FIRST algorithm, allowing us to account for the unique folding environment of membrane proteins. These studies provide a breakthrough in the understanding of the folding of these light-harvesting proteins. Analysis of several light-harvesting systems with FIRST indicates that (bacterio)chlorophyll plays an important role as a hydrogen-bond acceptor, linking transmembrane helices together into larger folding domains and constraining the conformational space available to loop regions.

1. Jordan, P., Fromme, P., Witt, H. T., Klukas, O., Saenger, W., and Krauss, N. (2001) *Nature* 411, 909-917
2. Liu, Z., Yan, H., Wang, K., Kuang, T., Zhang, J., Gui, L., An, X., and Chang, W. (2004) *Nature* 428, 287-292
3. McLuskey, K., Prince, S. M., Cogdell, R. J., and Isaacs, N. W. (2001) *Biochemistry* 40, 8783-8789
4. Papiz, M. Z., Prince, S. M., Howard, T., Cogdell, R. J., and Isaacs, N. W. (2003) *J Mol Biol* 326, 1523-1538
5. Jacobs, D. J., Rader, A. J., Kuhn, L. A., and Thorpe, M. F. (2001) *Proteins* 44, 150-165
6. Hesperheide, B. M., Rader, A. J., Thorpe, M. F., and Kuhn, L. A. (2002) *Journal of Molecular Graphics & Modelling* 21, 195-207
7. Hesperheide, B. M., Jacobs, D. J., and Thorpe, M. F. (2004) *Journal of Physics-Condensed Matter* 16, S5055-S5064

P17 Nonequilibrium Molecular Dynamics Simulation Study of Ion Currents through the KcsA Potassium Channel

Hendrick W. de Haan, Igor Tolokh, Saul Goldman, Chris Gray (*Guelph-Waterloo Physics Institute, University of Guelph*)

We have developed a nonequilibrium molecular dynamics technique which allows us to effectively simulate the transfer of ions through membrane channels by selectively applying an external voltage. This approach has been used on a system comprised of an atomic level model of the KcsA channel and explicit water molecules. From these simulations of ion currents, we have been able to calculate a current-voltage relationship for the KcsA channel which yields a channel conductance that is in good agreement with experimental results. Analysis of the trajectories has also given insight into the details of ion permeation through the selectivity filter as a highly cooperative process.

P18 A study of Bax oligomerization in solution and on the membrane of a giant unilamellar vesicle

Dmitri Satsoura, Brian Leber, David W. Andrews, Cecile Fradin (*Department of Biochemistry and Biomedical Sciences, McMaster University*)

Pore formation at mitochondria is one of the fundamental processes that regulate apoptosis. Bcl-2 family proteins play a pivotal role in both the regulation and the formation of large pores in the outer mitochondrial membrane. In particular, oligomerization of the pro-apoptotic protein Bax is believed to result in the formation of pores in the outer membrane of mitochondria. Determining the molecular mobility and oligomerization state of this protein is essential for understanding the molecular mechanism of action of Bcl-2 family proteins. The molecular mobility of a protein is expected to decrease when it interacts with membranes or if it binds to other proteins or oligomerizes. Molecular brightness, however, is expected to increase only upon oligomerization. The molecular brightness and mobility of Bax was assessed in the presence of a peptide that can trigger Bax insertion into artificial membranes, both in the absence and in the presence of membranes. Traditionally, protein-membrane interactions have been studied using small or large unilamellar vesicles. In this study however, we used giant unilamellar vesicles (GUVs), which due to their large size have minimum curvature of the membrane and therefore are better models for mitochondrial membrane. 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.

P19 **Interactions of Amyloid-Beta (1-42) Peptide with Model Lipid Membranes Followed using Atomic Force Microscopy**

Amira Choucair, Linda Johnston (*National Research Council, Steacie Institute for Molecular Sciences*)

Alzheimer's disease (AD) is characterized by the extracellular deposition of insoluble fibrils composed of the amyloid-beta peptide (A-beta). A-beta is a 39 to 43 amino acid peptide produced from the enzymatic cleavage of a transmembrane protein known as amyloid precursor protein. Previous studies suggest that the extracellular deposits of A-beta are responsible for the neurodegenerative changes observed in AD patients; however, the exact mechanism of action is still not clear. In the present study, atomic force microscopy (AFM) is used to follow the interaction of A-beta with lipid bilayers that mimic biological membranes. Two different lipid mixtures were used to prepare the bilayers. The first is a (1:1) mixture of dioleoylphosphatidylcholine and dipalmitoylphosphatidylcholine, which are lipids commonly found in plasma membranes. The second is a (2:2:1) mixture of dioleoylphosphatidylcholine, Egg-sphingomyelin and cholesterol, which is a model system for raft formation. Since gangliosides are abundant components of neuronal membranes, bilayers prepared in the presence of GM-1 ganglioside were also studied. AFM images show that both lipid mixtures undergo phase separation and form ordered microdomains. A-beta was found to preferentially accumulate on the ordered domains, which are rich in saturated lipids, sphingomyelin and cholesterol. After ca. 48 hours of incubation with the peptide, the bilayers began to show signs of damage, the extent of which increased with time. The degradation of the bilayer could be a possible pathway of A-beta neurodegenerative action.

P20 **Investigating the Photophysical Properties and the Diffusion of EGFP *in Vitro* and *in Vivo***

Asmahan A. Abu-Arish, Cecile Fradin (*McMaster University*)

The enhanced green fluorescent protein (EGFP), a mutant of the green fluorescent protein, is a widely used fluorescent probe for investigating protein-protein interactions, macromolecular dynamics, as well as protein function *in vivo*. For its importance, I am studying its dynamics and photophysical properties *in vitro* and in living cells. This study will be the starting point for studying the cellular dynamics of other less characterized proteins, such as the protein Ran that plays an important role in the process of nuclear transport in eukaryotic cells. The most striking photophysical property of EGFP is its blinking. Upon EGFP protonation (binding to a proton), the protein enters a non fluorescent state, and due to repetitive protonation and deprotonation, the protein is blinking. This blinking behavior can be characterized using fluorescence correlation spectroscopy, a technique that relies on the analysis of fluorescence fluctuations in order to study photophysical properties and dynamics of molecules. I checked that the relaxation time of the protonation state (blinking) depends on the pH of the solution. I also investigated the temperature influence on the blinking process. The blinking-temperature dependence can be used to characterize the structural conformational changes of the protein caused by heating. After introducing EGFP to living cells, I found that it diffuses almost freely in the cell without strongly interacting with other cellular molecules, which makes it a good control for the study of the mobility of other cellular proteins.

P21 **SNIFTIRS studies on the Adsorption of the Model Anionic Surfactant Sodium Dodecyl Sulfate (SDS) at the Au (111) electrode surface**

John J. Leitch, I. Burgess, J. Kunze, J. Li, J. Lipkowski, V. Zamlynny, J. Collins, A. Friedrich, U. Stimming (*Department of Chemistry, University of Guelph*)

Sodium Dodecyl Sulfate (SDS) is an anionic surfactant that has a polar sulfate head group attached to a 12-carbon hydrocarbon tail. As a result of the amphiphilic nature of the molecule two orientations have been observed on the Au(111) electrode surface depending on the applied potential. A hemimicellar cylinder conformation is observed at potentials between $-300 \text{ mV} < E < 400 \text{ mV}$ and an interdigitated conformation for potentials $> 400 \text{ mV}$. Subtractively Normalized Interfacial Fourier Transform Infrared Spectroscopy (SNIFTIRS) measurements can be used to study the effects of potential on surfactant molecules. The tilt angle (relative to the surface normal) for both the hemimicellar cylinder and indigitated conformations of SDS can be determined by examining the changes in IR intensity with respect to potential. The change in the tilt angle can then be determined from the interaction of the electric field and the net dipole moment of the adsorbed SDS molecule.

P22 PATTERNING IN 2D AND 3D WITH MICROMOULDING IN CAPILLARIES

Joe C. T. Leung, M. Cynthia Goh (*University of Toronto*)

Micromoulding in Capillaries (MIMIC) is a soft lithography technique that involves the spontaneous filling of channels formed by a mould on a substrate with capillary forces. Molecules that precipitate out of the solution adsorb on to the substrate, replicating the pattern of the mould; or, molecules can be left as residue on the substrate after the solvent evaporates. The choice of molecules, substrates, and mould materials can be quite broad in coping with factors that may facilitate or deter the channelling process such as electrostatic forces and hydrophobicity. Using a mould constructed of poly[dimethylsiloxane] we have demonstrated that it is possible to pattern small molecules, polymer chains, and spherical particles with a diameter of 1 μm directly on to glass. Using MIMIC indirectly, it was possible to pattern larger particles with dimensions greater than the channels. Although the microfabricated patterns did not form a perfect monolayer, the patterns achieved were able to diffract red and green light. This poster will show results from work with microspheres and polyelectrolytes using MIMIC, and a modified version of MIMIC incorporating the buildup of polyelectrolyte multilayers.

P23 Probing Protein-ligand Association with Free Energy Simulations in Four Spatial Dimensions

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

Novel computational techniques for the calculation of excess chemical potentials and binding free energies in full-atomic systems of biological scale are explored. An imaginary fourth spatial dimension is introduced as a computationally efficient means of turning on or turning off non-bonded interactions. Molecular simulations are used to compute the potential of mean force (PMF) acting on the molecule of interest in the fourth dimension. The excess chemical potential of that molecule is obtained as the difference in the PMF between fully-coupled and fully-decoupled systems. Direct calculation of the mean force by thermodynamic integration is shown to be more efficient and better suited than umbrella sampling, and affords a convenient way to analyze statistical error and optimize sampling efficiency. The generality, effectiveness, and reliability of the method are demonstrated in the calculation of the hydration free energy of polar, non-polar, and ionic species. The method is applied to the calculation of the absolute binding free energies of benzene and other ligands to the L99A engineered variant of T4 Lysozyme. Results are corrected for standard state conditions and compared directly to experimental measurements.

P24 Investigating Magnetically Functionalized Paclitaxel

Allan R. MacDairmid, Torin Huzil, Mark Freeman, and Jack Tuszynski (*Department of Physics, University of Alberta*)

In an effort to enhance our ability to manipulate and align microtubules in solution, we are currently studying a variety of paclitaxel derivatives that have magnetic domains. The ability of the functionalized paclitaxel molecules to bind and stabilize microtubules is verified by monitoring the absorption at 340 nm. Stability is determined in comparison to microtubule assembly in the presence and absence of commercially available paclitaxel. We will discuss initial investigations into the magnetic properties of both the modified paclitaxel and the subsequently assembled microtubules by circular dichroism measurements. Possible applications will also be discussed.

P25 Local Deformation of the Red Blood Cell Membrane Caused by Focused Laser Beam Illumination

Felix H.C. Wong, Cecile Fradin (*McMaster University, Department of Physics & Astronomy*)

We are investigating the rapid formation of a small imprint on the membrane of red blood cells (RBCs) upon illumination by a focused laser beam in the presence of an external fluorophore. This morphological change of the membrane appears to be the very first step of the well-known photohemolysis process which is exploited in photodynamic therapy. We show that when a laser beam is focused on the membrane of a RBC, the membrane is pulled toward the inside of the cell, independent of the direction in which the light is traveling. We do not observe imprint formation on the lipid membrane of vesicles nor on the membrane of nucleated mammalian cells such as HeLa cells, meaning that this effect is specific to RBCs, and probably due to the unique structure of the RBC membrane and its underlying actin/spectrin cytoskeleton. We found that the rate of the imprint formation depends on laser input power, fluorophore concentration, and the presence of oxygen scavenger, but that it is independent of the buffer pH value. These observations suggest that the imprint is formed via oxidation of RBCs membrane proteins. They confirm that membrane proteins play a pivotal role in the preservation of the characteristic RBC shape.

P26 **Nanomechanical Investigation of Bacterial Cellulose fibers using Atomic Force Microscopy**

Ganesh Guhados, Jeffrey L. Hutter, W. K. Wan (*The University of Western Ontario*)

Bacterial cellulose (BC) is produced extracellularly by the bacterium *Acetobacter xylinum* BPR2001 in the form of fine fibers of diameter < 100 nm. It has a high degree of crystallinity and is used in the preparation of biocompatible nanocomposites, wound dressings and small diameter blood vessels. Although the mechanical properties of BC have been studied on the macro-scale, the nanomechanical properties of individual fibers are not clearly known. The ability of Atomic Force Microscope (AFM) to measure nN forces has led to its use to study the mechanical properties of nanomaterials such as carbon nanotubes and poly-pyrrole nanotubes. Previously, a known force was applied at a single point near the centre of a suspended tube and the elastic modulus was deduced from an assumed elastic model. We extend this technique by performing a nano-scale three point bending test at a series of points along the suspended fiber to establish a more accurate modulus value and allows us to verify the model used. Also we find that a single measurement at a point near the center of the fiber can result in a disproportionately large error in the measured Young's modulus. Thus it is critical to collect data at different points on the fiber. We determine an accurate elastic modulus of 78 ± 17 GPa for BC fibers of diameter ranging from 35-90 nm. This value is considerably higher than previous results and is consistent with the Voigt model for the parallel arrangement of the crystalline regions within the fiber.

P27 Coherent Control of Population Transfer in Rhodamine 101 Using Tailored Light Pulses

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

We performed a series of experiments for the optimization of the population transfer from the ground to the first excited state in rhodamine 101 solvated in methanol, using shaped excitation pulses. The pulses were tailored using an acousto-optic programmable modulator which allows for independent control of both phase and amplitude, and pulse characterization was carried out using a transient grating frequency resolved optical gating setup. The optimal pulse shape for a given experimental observable such as differential absorption, which reflects the population of the excited electronic state, is found using a genetic algorithm. The experiments are performed at very low intensities (1 absorbed photon per 100-500 molecules per pulse). The spectrum of the optimal excitation pulse, which results in an excited state population enhancement of $\sim 15\%$, consists of a series of well-resolved peaks spaced apart by ~ 6.5 nm, corresponding to a frequency of 220 cm^{-1} . This frequency matches well with the frequency of beatings (period of ~ 150 fs) in the population kinetics, observed by impulsive excitation using a short (~ 20 fs) transform-limited laser pulse. Anti-optimization experiments performed under the same conditions yield a 30% decrease in the population transfer compared to the optimal pulse shape, indicating success of our control scheme.

P28 Protein interaction quantification by spatially and spectrally resolved fluorescence resonance energy transfer

Russell Fung (a), Vali Raicu (a), Dilano K. Saldin (a), David B. Jansma (b), James D. Friesen (b) (*(a) University of Wisconsin-Milwaukee, (b) University of Toronto*)

Spectrally-resolved Fluorescence Resonance Energy Transfer (FRET) is a technique for studying protein interactions in vivo, whereby fluorescence spectra are acquired of fluorescently-tagged protein molecules of interest. Two types of tags are used, which are chosen so that the emission spectrum of one (donor) overlaps strongly with the absorption spectrum of the other (acceptor). Depending on the particular application, donor and acceptor tags could be attached either to the same type of protein or two different types of protein. Upon excitation with laser energy close to the absorption peak of the donor, acceptor tags fluoresce if they are in close proximity of donor tags and thus are able to 'steal' energy from the donor tags (in addition to direct excitation by the laser). Spectral analysis of fluorescence images from such experiments thus gives information about the relative concentrations of the two types of proteins and their molecular complexes. Fully quantitative FRET is currently spectrally but not spatially resolved, that is, parameters characterizing protein interactions are only obtained as averages over some cellular area or volume. Doing FRET on a pixel level allows one to gain more detailed information about protein interactions, but also presents mathematical and conceptual challenges. This poster will give a brief overview of our theoretical advances in pixel-level FRET, and will present some preliminary results.

P29 **Simulations of Proton Transport in Water Using the Multistate Empirical Valence Bond Model**

Marty Kurylowicz, Regis Pomes (*University of Toronto and Hospital for Sick Children*)

The Multistate Empirical Valence Bond (MS-EVB) model offers a hybrid quantum/classical treatment of proton transport in water, as a delocalized process over several hydration shells. The model constructs a superposition of many possible bond states among a cluster of water molecules with an excess proton, reorganizing the bond arrangements to represent a hydronium cation centred on various oxygen atoms within the cluster. The Hamiltonian for each state is written strictly as a function of the nuclear degrees of freedom, capturing the electronic behaviour by means of empirical force field parameters as used in standard Molecular Dynamics (MD) simulations. The quantum character of the problem is captured by constructing a Hamiltonian matrix from these states that includes terms representing the interaction between simultaneous states; solving this matrix yields the lowest energy placement of the excess proton, and the system's motion is then updated using MD. We are implementing this model in CHARMM, with the aim of studying proton transport along hydrogen-bonded networks of water molecules in biological systems such as enzymes and channels.

P30 **Simulating the kinetics of a simplified polypeptide model: what can it tell us about protein cooperativity?**

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

Many small globular proteins fold in an apparently two-state manner, displaying the experimentally observed properties of thermodynamic and kinetic cooperativity. We model these phenomena computationally, using Langevin dynamics simulations to investigate the folding of a simplified atomic model of a 54-residue polypeptide which is designed to fold to a three-helix bundle. The model is continuous (off-lattice) and represents all the backbone atoms together with the H and O side atoms attached, respectively, to the N and C' backbone atoms; the side groups are represented as single C_β atoms. The potential energy function, which is non-Gō-like (i.e., not biased towards the target structure), contains representations of hydrophobic and hydrogen bonding forces. To our knowledge, this is the first time that a protein model with this level of detail has been tested for kinetic cooperativity. The chevron plots for folding and unfolding display marked rollovers; this departure from kinetic cooperativity is consistent with the model's relatively low level of thermodynamic cooperativity. The simulation results lead us towards a more general theoretical understanding of (i) the energetics that underlies thermodynamic and kinetic cooperativity, and (ii) the role that cooperativity often plays in the folding process of small globular proteins.

P31 **Molecular Dynamics Simulations of the Structure and Mechanical Properties of Elastin**

Sarah Mansour, Stephanie Baud, Regis Pomes (*University of Toronto and Hospital for Sick Children*)

The function of much of the biological machinery within the body is dependent on its ability to endure complex dynamic changes. Elastin, together with other structural proteins, forms the fabric of connective tissues, providing the propensity for elastic recoil. Elastin also undergoes an inverse temperature transition in which it becomes more ordered upon heating. In vitro, elastin and elastin derived peptides are observed to coacervate, forming an organized fibrillar structure. Elastin therefore has a tendency to self-aggregate and self-organize, making elastin derived peptides ideal candidates for biomaterials applications, such as tissue replacement.^{1,2}

In order to elucidate the chemical and physical basis of these properties, molecular dynamics (MD) simulations are being conducted on units from the hydrophobic domains of tropoelastin. Previous MD studies have provided a number of explanations for the origin of elastin's properties. However, the relevance of these simulations must be questioned due to limited statistical sampling and small system sizes.^{3,4,5,6} A new, and more computationally efficient, approach is used. Replica exchange free energy perturbation (REFEP)⁷ is used to reveal the free energy profile between stretched and relaxed states of elastin. Multiple replicas of elastin fragments with differing end-to-end lengths are simulated simultaneously in the isothermal-isobaric ensemble, using the CHARMM force field. Swapping moves occur periodically between replicas according to the Metropolis Monte Carlo criteria, which has been shown to dramatically improve configurational sampling.⁷ The goal of these simulations is to obtain a more refined structural and thermodynamic description of the elasticity and coacervation of elastin.

1. Bellingham, C. et al. 2004. *Current Opinion in Solid State and Materials Science* **8**, 135-139.
2. Keeley, F. et al. 2002. *Phil. Trans. R. Soc. Lond. B.* **357**, 185-189.
3. Floquet, N. et al. 2004. *Biopolymers.* **76**. 266-280.
4. Urry, D. et al. 2002. *Journal of Muscle Research and Cell Motility.* **23**. 543-559.
5. Li et al. 2001. *J. Amer. Chem. Soc.* **123**. 11991-11998.
6. Schreiner, E. et al. 2004. *Phys. Rev. Lett.* **92**. 148101-1 – 148101-4.
7. Woods, C. et al. 2003. *J. Phys. Chem. B* **107**. 13703-13710

P32 Measuring the Effects of Stimulation on Cell-Protein Interactions Using the Atomic Force Microscope

Stephen D. Hudson, Jeffrey L. Hutter, Bosco M. C. Chan, Shashi Uniyal, Wankei Wan (*The University of Western Ontario*)

Adhesion to the extra-cellular matrix (ECM) is critical to cell proliferation, motion, and survival. Using the atomic force microscope (AFM), we have studied the effect of the known stimulant phorbol 12-myristate 13-acetate (PMA) on the interaction between the integrin-ligand pair of $\alpha_5\beta_1$ integrin (cell receptor) and fibronectin (ECM protein), in NIH-3T3 mouse fibroblast cells. By making force measurements with a bead functionalized with fibronectin and attached to an AFM cantilever, we measured bond strength, probability of bond formation, and the elastic modulus of the cells. Bond strength was of the order 100 pN both before and after stimulation. The probability of bond formation increased dramatically from 6% before stimulation with PMA to 29% after stimulation. Analysis of the force measurements revealed no correlation in time, indicating that the bond formation is a random process. Measurements of the elastic modulus (typically 2–4 kPa for these cells) showed no correlation with bond formation, suggesting that the bonding is independent of local structure, or time, indicating that there was no measurable mechanical stimulation due to our functionalized cantilever.

P33 Protein aggregation: Collagen self-assembly in silico

Ivan Vinogradov, Darren J. Anderson, Pedro Borkowski, M. Cynthia Goh (*University of Toronto, Department of Chemistry*)

Collagen is an abundant extra-cellular protein with a unique triple helical structure and an ability to self-assemble into several different aggregates. The understanding of this process has been impeded by the lack of a complete 3D structure, impossible to obtain using current experimental techniques. Therefore, a model of human type I collagen was created containing both backbone and side-chain atoms using a reduced rotamer representation derived from a statistical analysis of previously solved structures. Minimization was performed using molecular mechanics and dynamics simulations both in-vacuo and with water as solvent. The final structure provides information about detailed geometry of the monomer unit including bulk properties and surface charge distribution.

P34 **A Real-time Observation of the Disassociation of Rec/DNA Complex**

Bing Shi Li, Cynthia Goh (*Department of Chemistry, University of Toronto*)

RecA protein plays a pivotal role in the promotion of both DNA pairing and the strand exchange reaction. Binding of RecA to DNA is considered to be a multiple step process that requires the cofactors Mg^{2+} , ATP or ATP γ . In this work, we demonstrated the important role of these cofactors in stabilizing the RecA/DNA complex. In the presence of these cofactors RecA was found to bind to dsDNA, giving rise to the formation of the helical fiber-like assemblies; however, in the absence of the cofactors the complex underwent a disassociation process. By using an intermittent contact mode AFM equipped with fluid cell, we observed the disassociation process of the RecA/DNA complex in solution in real time. This disassociation was observed to initiate from multiple distinct sites of the RecA /DNA complex. The disassociation rate was also derived.

P35 **Constant enthalpic barriers to protein folding under isostability conditions: effect of hydrophobic interactions**

Zhirong Liu, Hue Sun Chan (*Department of Biochemistry, University of Toronto*)

The temperature dependence of protein folding rate in water is typically non-Arrhenius, i.e., it shows a nonlinear dependence of logarithmic rate on $1/T$. However, when the system is controlled under isostability conditions by adjusting both temperature and denaturant concentration, it has been demonstrated in a few proteins that the folding rate is essentially Arrhenius. In this work, by use of experimental data in the literature, we verify the Arrhenius behaviors of 14 proteins under isostability conditions and determine their intrinsic enthalpic folding barriers, which vary from 15 to 40 kcal/mol for different proteins. The enthalpic barriers do not appear to correlate with folding rates. An explicit-chain model with temperature-dependence of hydrophobic interaction is adopted to investigate the protein folding via molecular dynamics simulation. It is shown that the small microscopic desolvation barriers with entropy-enthalpy compensation can act cooperatively to give rise to a significant overall enthalpic barrier to protein folding.

P36 **Anomalous diffusion due to molecular crowding in model systems and in living cells studied by fluorescence correlation spectroscopy**

Daniel Banks, Asmahan Abu-Arish, Cécile Fradin (*McMaster University, Dept. of Physics and Astronomy*)

The diffusion of proteins in living cells has sometimes been reported to be anomalous, with a mean square displacement $\langle r^2 \rangle \sim t^\alpha$ and $\alpha < 1$. This effect is still poorly understood and controversial. One possible explanation for this behavior is the crowding of the cellular environment. To test this hypothesis, we have studied the influence of molecular crowding on the nature of the diffusion in model systems. We used fluorescence correlation spectroscopy to study the mobility of different tracer molecules in solutions containing increasing concentrations of dextrans, up to 400g/l. We observed that for very small tracers such as dyes the diffusion is normal ($\alpha = 1$), but that for proteins the diffusion is anomalous, with the exponent α as low as 0.75 at high polymer concentrations. At low polymer concentrations, the value of α is highly dependent on the polymer molecular weight. The anomalous diffusion of the tracer proteins was also observed when other proteins were used as obstacles instead of the dextrans. We propose that two mechanisms participate in making the diffusion anomalous in our system: at low dextran concentration collective rearrangements of particles, and at high dextran concentration the formation of an entangled network. Measurements of the diffusion of the green fluorescent protein in the nucleus of living cells confirms that it is undergoing anomalous diffusion with $\alpha \sim 0.75$, supporting the hypothesis that molecular crowding might play an important part in making cellular protein diffusion anomalous.

P37 **Effects of Solute Size and Guanidinium Salt Concentration on Hydrophobic Association**

Christopher A Madill, Tomas Rodinger, Hue Sun Chan, Seishi Shimizu, Régis Pomès, Grace Li (*Hospital for Sick Children*)

Although salt effects are an important aspect of protein stability, they are not well understood at the molecular level. In order to probe the fundamental physical basis for the salt dependence of hydrophobic association, we examine the thermodynamic properties governing the interaction of two simple hydrophobic solutes at various salt concentrations using molecular dynamics simulations. The potential of mean force (PMF) for the association of two Lennard-Jones spheres mimicking methane and neopentane solutes is computed in aqueous solutions of guanidinium hydrochloride (GuCl) ranging from 0 to 5M. Absolute free energies of hydration and of contact pair formation are computed to allow a direct comparison of PMF profiles as a function of ionic strength. The salt and size dependence of hydrophobic association are analyzed in terms of solute and ionic hydration.

P38 **Polarization Modulation Infrared Reflection Absorption Spectroscopy: A powerful technique to study biomimetic membranes**

Thamara A. Laredo, Xiaomin Bin, John R. Dutcher, Jacek Lipkowski (*Department of Chemistry*)

Owing to the intrinsic field across biological membranes, electrochemistry provides an ideal means to study electric field driven processes in supported lipid bilayers. Recent studies have been aimed at closely resembling real cell membranes using phospholipids as the base matrix with the addition of cholesterol, proteins and carbohydrates in biomimetic proportions. The need for non-perturbing techniques that allow *in-situ* analysis of the physical properties and conformation of the elements that form these kinds of systems is of both physical and biological interest. Fourier transform infrared spectroscopy (FTIR) has been widely used for the study of biological molecules since the shape, intensity and position of the infrared bands are sensitive to the structure and motion of the molecular species. In addition, due to the fact that the absorption of the IR bands depend on the angle between the polarization of the incident radiation and the transition moment of a given vibration, it is also possible to characterize the molecular orientation in samples deposited on a substrate by means of polarized FTIR. The present work is done under a controlled electric field of similar magnitude to that existing in real biological systems and the orientation and conformational changes driven by this electric field are studied in different types of model membranes by means of polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS). PM-IRRAS has proven to be a powerful technique to quantitatively study orientation and conformation changes in phospholipid bilayer samples containing as little as 10% of Gramicidin, a channel forming membrane protein.

P39 **Charge Renormalization and Inversion of a Highly-Charged Lipid Bilayer: Effects of Dielectric Discontinuities and Charge Correlations**

Sattar Taheri-Araghi, Bae-Yeun Ha (*Physics Department, University of Waterloo*)

We study the charge renormalization and inversion of a highly-charged lipid bilayer in monovalent (e.g., NaCl) and ($Z : 1$) (e.g., CaCl_2) electrolytes. In particular, we study how charge correlations and dielectric discontinuities influence the 'effective' charge of the bilayer. We find that, while both effects enhance counterion condensation (adsorption) onto the bilayer, the correlation effect is more pronounced when the bilayer has a lower dielectric constant, inverting the sign of the effective charge at a smaller value of Ca^{2+} concentration. This finding contradicts the earlier observation that image charges weaken the attraction of counterions to an oppositely charged surface of a low dielectric constant. With a simple scaling picture, we show how image-charge effects and backbone-charge distributions are intertwined: Image charges diminish condensation if the backbone charge is uniformly smeared out while counterions are localized in space; they can, however, enhance condensation when the backbone charge is discrete.

P40 **The Role of Water in Structural Transitions of Biophysical Systems**

Susan Csiszar and Jeremy Schofield (*Department of Chemistry, University of Toronto*)

A discontinuous molecular dynamics (DMD) simulation of water is presented in order to form the basis for a future investigation of solvent effects in biomolecular systems. The structure and folding of a solvated protein is partly determined by the interactions of the protein molecules with the water molecules. In order to create a reasonable model of a protein's folding and structure, solvent effects need to be explicitly included. To date these solvent effects have been studied using continuous potential molecular dynamics (MD) models that describe the interactions in the system. MD models can be quite computationally demanding for large systems or long time scales and we have devised a new water model using DMD which is more efficient than continuous MD methods. The goals of our approach are to describe the local structure and thermodynamics of water in a semi-quantitative fashion.

P41 **Using Computer Modeling to predict Collagen SLS Assembly**

Pedro Borkowski, Darren Anderson, Ivan Vinogradov, M. Cynthia Goh (*Department of Chemistry, University of Toronto*)

Collagen is the most abundant protein in the human body. The usual aggregation of collagen is fibrillar. Once Adenosine Triphosphate (ATP) is added to collagen, Segmental Long Spacing (SLS) collagen crystallites are formed. The aggregation of SLS collagen is not well understood, however, studies have shown that ATP interacts with collagen monomers to produce crosslink ATP-collagen crystallite. Our study investigates the assembly of SLS aggregates. We employ molecular dynamics and molecular mechanics that use the CHARMM27 force fields to make predictions of our system. It was found that the ATP phosphates attach to the basic amino acids in the collagen monomers and the nucleotide ring attach to the acidic amino acids in the collagen monomer. These predictions indicate a mechanism that would allow the full aggregation of the collagen crystallite.

P42 **Probing Hydration of Counterions in the Vicinity of Nucleic Acids**

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

This study is aimed at investigating the hydration properties of univalent counterions in the vicinity of nucleic acids. To this end, we use densimetric and ultrasonic velocimetric measurements to evaluate, at 25 C, the partial molar volumes and adiabatic compressibilities of the poly(dAdT)poly(dAdT), poly(dGdC)poly(dGdC), poly(dIdC)poly(dIdC), poly(rA)poly(rU), poly(rG)poly(rC), poly(rI)poly(rC), and 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 structure was exhaustively 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 (LiCl, NaCl, KCl, RbCl, CsCl, NH₄Cl, and N(CH₃)₄Cl) plotted versus the partial molar volume/adiabatic compressibility of the salt should yield a straight line with a slope of unity. Any deviation from such a line is suggestive of DNA influencing counterion hydration. Our analysis reveals that the poly(dGdC)poly(dGdC), poly(dIdC)poly(dIdC), and poly(rI)poly(rC) duplexes and single-stranded poly(rU) do not significantly influence the hydration properties of their condensed counterions. In the vicinity of these polymers, counterions retain their full hydration shells (within 15%). By contrast, counterions condensed around the poly(dAdT)poly(dAdT), poly(rA)poly(rU), and poly(rG)poly(rC) duplexes are significantly dehydrated and retain, respectively, only 65±18%, 34±21%, and 33±9% of their original hydration shells. Taken together, the volumetric data reported here provide important new information that ultimately may help us understand the central role that hydration and counterions play in modulating the conformational preferences of nucleic acids and the energetics of DNA recognition events.

P43 **Quantum-classical dynamics of nonadiabatic proton transfer**

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

Proton transfer is of great importance to many processes in chemistry and biology. Studies of proton transfer in the condensed phase require that one considers the dynamics of a quantum subsystem coupled to a classical bath. An approach to studying such a composite system is quantum-classical molecular dynamics. The main idea behind this approach 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 model for a proton transfer reaction in a hydrogen-bonded complex, which is dissolved in a polar solvent, is simulated using both adiabatic and nonadiabatic dynamics. We investigate the role of coherently coupled adiabatic surfaces and decoherence in determining the mechanism and rate of condensed phase proton transfer processes.

P44 **Revelation on the kinetic parameters of Tripartite Molecular Beacons during conformational changes.**

Nelson Chen, Cecile Fradin (*McMaster University*)

Detection of nucleic acid has improved tremendously over the past decade. One important step was the introduction of Molecular Beacons (MBs), which are single stranded DNA molecules with a hairpin structure that open up and emit a fluorescence signal in presence of their target nucleic acid sequence. We are studying the properties of slightly modified beacons called Tripartite Molecular Beacons (TMBs) as they are composed of three ssDNA sequences. TMBs are advantageous in that they are cheaper to produce and have a higher design flexibility. In order to perform as well as MBs in terms of nucleic acid detection, TMBs need to have similar kinetic parameters, i.e. the rate constants for opening and closing of the hairpin need to be similar. We used a method based on fluorescence correlation spectroscopy (FCS) to measure these rate constants for a MB and TMBs with stems of different length and found that to achieve similar kinetic properties the TMBs need to have a stem one base-pair longer than the MBs. We also investigated whether binding of the TMB to a streptavidin molecule, through a biotin/avidin link would modify the kinetic properties of the DNA molecule. Indeed, we found that the relaxation time associated with the opening and closing of the beacon increased upon binding to streptavidin. This indicates that for *in vivo* applications, for which it was shown that binding to streptavidin was essential in order to maintain a cytoplasmic localization of the beacon, different beacon designs could be helpful.

P45 **Study of Domain Dynamics in Lipid Bilayers and Cell Membranes**

Vladimir Smorodin¹, Anja Nohe² (1-Dept. of Chem. Eng., University of Utah, SLC, UT 84101; 2-Institute of Molecular Biophysics, Dept. of Chem. & Biol Eng., The University of Maine, Orono)

Nanostructures, e.g. caveolae, coated pits and lipid rafts in cell membranes play crucial roles in the cell ion-exchange, bioenergetics, cell metabolism, adaptation, and survival. We used the Family of Image Correlation Spectroscopy and single particle tracking to investigate the dynamics of membrane domains by studying the temperature dependent movement of marker proteins present in these domains in living cells. Our data show that the domains have different diffusion behaviour and temperature dependence. Lipid bilayers can be regarded as relevant systems to model the real cell membranes. In our presentation the results of theoretical investigations of stability and evolution of gel-domains in mixtures of the DMPC/DSPC phospholipid bilayers are reviewed. The analysis is based on a dependence of the Gibbs free energy of the non-charged polarized domains on its shape variations and main regulating physico-chemical parameters. Critical size of the domains corresponding to the domain shape transitions and fission are calculated. The difference in dimension of gel-domains in phospholipid bilayers comparing with those in monolayers is explained, ascribing it to the interaction of the polarized surface of the two opposing domains pertaining to the two bilayer leaflets. Theoretical results on domain dynamics were found in a good agreement with experimental data obtained by the FRAP method and their Monte-Carlo simulation.

We believe this new conceptual approach establishes a perfect background for further investigating the domain dynamics in artificial and real membranes.

P46 **Structural order in complexes of DNA with some HMGB-domain proteins**

Alexander M. Polyanichko, Zoya Leonenko, Elena Chikhirzhina, Helmut Wieser
(*Department of Chemistry, University of Calgary*)

HMGB-domain proteins constitute a family of non-histone chromosomal proteins known as ‘architectural factors’ of chromatin. Each protein in the family contains at least one structure-function DNA-binding domain called HMGB-domain. The family is widely known by members such as HMGB1, LEF-1, SRY, UBF and TCF-1. In addition to HMGB-domains, some of the proteins contain C-terminal sequences of tens of Asp and Glu amino acid residues, which are believed to be modulators of the proteins’ DNA binding activity. It was shown earlier that the HMGB-domain sequence is able to form highly ordered complexes with DNA. Optical properties of such complexes were shown to be similar to those of DNA liquid crystals. Our present study aims to reveal structural organization of these DNA protein complexes. Using a combination of circular dichroism, fluorescence and atomic force microscopy we have shown that the structural organization of the complexes depends on ionic strength and protein to DNA ratio (\mathbf{r}) in the complex, but most of all on the presence or absence the C-terminal acidic sequence. We have demonstrated that regardless of the presence of the Asp/Glu sequence the proteins induce remarkable DNA compaction. However, a protein lacking this C-terminal domain induces more considerable structural distortions in DNA and at some critical \mathbf{r} induces the formation of highly ordered DNA-protein complexes revealed by AFM images.

P47 **HMGB1/H1 interaction with DNA: Working as a Team**

Alexander M. Polyanichko, Helmut Wieser (*Department of Chemistry, University of Calgary*)

Non-histone chromosomal protein HMGB1 is one of the most abundant non-histone proteins in chromatin. The exact role of the protein in chromatin functioning remains unclear, but there are strong reasons to believe that it plays an important role in the higher levels of chromatin structural organization. Histone H1 is another nuclear protein crucial for compacting nucleosomal DNA into 30 nm fiber. Upon interacting with DNA these proteins are known to induce considerable DNA condensation *in vitro*. However, the most commonly used structure-sensitive techniques such as UV circular dichroism (ECD) are not useful for such complexes due to the high level of light scattering of their solutions. To overcome that, we used a combination of IR absorption and vibrational CD (VCD). With this combination we were able to show that in such ternary complexes HMGB1 binds DNA in the minor groove, while H1 facilitate this binding by screening negative charges of both DNA and HMGB1 and interacting with phosphate groups of DNA and C-terminal sequence of Asp/Glu amino acid residues of HMGB1. The manner of the protein interactions is sensitive to the presence of divalent cations in solution, which is also discussed using Ca^{2+} and Mn^{2+} ions as examples.

P48 **A comparative analysis of Ca^{2+} and Mn^{2+} influence on HMGB1-DNA interaction.**

Alexander M. Polyanichko, Valery Andrushchenko, Elena Chikhirzhina, Helmut Wieser (*Department of Chemistry, University of Calgary*)

Electrostatic interactions play a very important role in the interaction of non-histone chromosomal protein HMGB1 with DNA. The mode of the HMGB1-DNA complexation is strongly affected by the presence in the protein structure of the negatively charged C-terminal sequence of 30 Asp and Glu amino acid residues. However, unusual DNA-binding properties of the protein are determined by the structural-functional motif called HMGB-domain. It was shown earlier that in some cases the presence of divalent cations in electrostatically negligible concentrations is crucial for protein functioning *in vivo*. To determine the effect of Mn^{2+} on the structure of DNA-protein complexes we chose Ca^{2+} and Mn^{2+} , which are known to interact differently with DNA. We applied a combination of absorption and circular dichroism spectroscopy in the UV and IR regions and have shown that both ions partially screen the negative charge of the C-terminal Asp/Glu sequence. However, the most important effect on the protein interaction with DNA arises from the ability of Ca^{2+} to bind HMGB-domain of the protein, changing the DNA-binding properties of the latter. At the same time, Mn^{2+} coordinates to DNA bases, changing the pattern of the protein binding sites on DNA. The results obtained showed that it is the specific interactions of the metal ions with the macromolecules which determine the local structural organization of the complexes.

P49 **Pressure Denaturation of Double-Stranded DNA**

Gamal Rayan, Robert B. Macgregor Jr. (*Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto*)

We have employed (spectroscopically monitored) elevated hydrostatic pressure to study the thermodynamics of the double-stranded DNA denaturation. Increased pressure can stabilize, destabilize or have no effect on the native state depending on the volume change (ΔV) of the helix-coil transition. A theoretical phase diagram for the helix-coil transition of double stranded nucleic acids predicts that the polymer whose melting temperature (T_m) is below $\sim 50^\circ\text{C}$ ($\Delta V < 0$) will be destabilized by pressure and can be pressure-denatured at isothermal conditions. Previously we were able to pressure denature Poly[d(AT)] whose $\Delta V < 0$. The ΔV of the pressure-induced denaturation was much greater than the ΔV for the heat-induced transition. Our current study has focused on the effects of pressure on Poly[d(IC)]. In agreement with the phase diagram (and the results obtained with Poly[d(AT)]) we were able to pressure-denature Poly[d(IC)] when $\Delta V < 0$. Similarly, the ΔV of the pressure-induced helix-coil transition was greater than that of the heat-induced transition. The mid-point of the pressure-induced helix-coil transition (P_m) increases with decreasing temperatures below the T_m .

P50 **Complete Kinetic Profiles for Biochemical Reactions Using an Electro-spray Coupled Capillary Micromixer: A Detailed Study of the Acid Induced Denaturation of Inducible Nitric Oxide Synthase**

Derek J. Wilson, Steven P. Rafferty, Lars Konermann (*University of Western Ontario*)

Electrospray ionization mass spectrometry (ESI-MS) has proven a powerful tool for kinetic studies on the time scale of minutes to hours. ESI-MS offers the advantage that virtually any analyte can be detected and distinguished, even in very complex solutions. However, the development of ESI-coupled rapid mixing devices for the detection of short-lived reaction intermediates has proven challenging. We have implemented a capillary micromixer with adjustable reaction chamber volume that can be coupled directly to the inlet of an ESI mass spectrometer. The device is used to obtain a series of hundreds or thousands of mass spectra that reflect the progress of a (bio)chemical solution-phase process with millisecond time resolution. From these spectra, detailed intensity-time profiles for any reactive species can be extracted. This technique has been used to obtain new mechanistic information for a number of biochemical reactions from small molecules to enzyme kinetics. Most recently, we have used the apparatus to study the acid induced dissociation and unfolding of the inducible nitric oxide synthase oxygenase domain. The denaturation mechanism exhibits an extremely complex behavior that includes several unfolding intermediates and non-native ligand binding states. Global analysis of the data indicates three reaction processes with relaxation times $\tau_1 = .36$ s, $\tau_2 = .62$ s and $\tau_3 = 3.3$ s.

P51 **Effect of Pressure on Hydrophobic Interactions**

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

High pressure studies of chemical reactions, solutions, and polymers have provided very useful information from both a fundamental and a practical point of view. Starting in 1914, high pressure has been used as a tool to study volume changes, formation of partially structured intermediates upon protein unfolding as well as dissociation of aggregated proteins. There are not, however, many studies regarding the effect of pressure on hydrophobic interactions which are considered one of the major driving forces in protein folding. As far as the hydrophobic effects are concerned, most of the effort in the scientific community has been focussed on the study of effect of temperature on hydrophobic interactions at atmospheric pressure. While these studies have offered remarkable insight where the effect of temperature is considered, they have not investigated whether the results obtained will be valid at pressures beyond 1 atm. Our understanding of the hydrophobic interactions cannot be considered complete without more work in the effect of pressure on hydrophobic interactions. In this work, we are examining the effect of pressure on hydration properties of a single methane-like solute. We will study all thermodynamic signatures and their pressure and temperature dependences with an emphasis on associated heat capacity changes.

P52 **Quantitative Analysis of mRNA using Gel-Free Capillary Electrophoresis Mediated by ssDNA-Binding Protein**

Azza A. Al-Mahrouki, Sergey N. Krylov (*York University*)

We introduce a highly sensitive and rapid method for quantifying specific mRNA in gel-free capillary electrophoresis with laser-induced fluorescence detection. The method is facilitated by replacing the gel with single-stranded DNA-binding protein (SSB), which is added to the run buffer. SSB binds ssDNA with much higher affinity than ssRNA or dsDNA or RNA/DNA hybrid. This unique feature was used in this work to efficiently separate the excess of the hybridization probe from the probe-mRNA hybrid. As a model we used mRNA of green fluorescent protein (GFP). A fluorescently labelled ssDNA probe, which can hybridize with the 3' end of the GFP mRNA, was used. The probe-mRNA complex had two regions: a short double stranded RNA/DNA hybrid and a long ssRNA overhang. SSB could not bind either of the two regions but could bind free ssDNA probe. While bound to the probe, SSB significantly shifted its mobility in electrophoresis. This differential electrophoretic mobility allowed us to efficiently separate the excess of the ssDNA probe from the probe-mRNA complex. The complex was then quantified with highly sensitive laser-induced fluorescence detection. To summarize, the method is direct, rapid, and sensitive. It does not involve error-prone RT-PCR and is extremely simple in performance as it does not require gels. The method addresses most of the reported limitations of the other approaches used to analyze mRNA. This method will be an indispensable analytical tool in quantitative analyses of mRNA.

P53 **Destruction of Spiral Waves in Chaotic Rossler Media**

Meng Zhan, Raymond Kapral (*Chemical Physics Theory Group, Department of Chemistry, University of Toronto*)

The destruction of spiral wave is studied in the Rossler reaction diffusion equation as the local attractor changes from a phase-coherent to a non-phase-coherent (funnel) form where the definition of a local phase is not always possible. Stable spiral waves are observed to persist even when the local chaotic attractor has a funnel structure. An analysis of the chaotic phase shows that the stability or destruction of funnel spiral waves in strongly chaotic media is determined by the competition between the Archimedean spatial structure of the funnel spiral and the local nonlinear chaotic dynamics.

P54 **Experimental coherent control of the retinal isomerization yield in bacteriorhodopsin**

Valentyn I. Prokhorenko(1), Leonid Brown(2), Andrea Nagy(1), R.J. Dwayne Miller(1) (*(1) Department of Chemistry, University of Toronto; (2) Department of Physics, University of Guelph*)

We present our recent experimental studies of the trans-cis isomerization control in bacteriorhodopsin (BR) performed under low excitation conditions, where only 1 of 20 retinal molecules absorbs a photon during the excitation cycle. Using specially shaped excitation pulses (found in optimization experiments using a genetic search algorithm) we are able to manipulate the 13-cis yield in BR over an absolute range of 40 % (20% enhancement as well as 20% suppression in comparison to excitation with a transform-limited pulse). The temporal structure of the optimal pulse, retrieved using a FROG-setup, shows a series of sub-pulses with a characteristic period of ~ 160 fs. The Wigner transform displays the presence of several oscillatory components that can be considered as the overtones of the main component with frequency of ~ 200 cm^{-1} . We conclude that such an excitation pulse selectively and efficiently drives a torsional mode in retinal (identified in previous Raman studies of BR) and thus enhances the isomerization yield. A comparison with previous experimental studies of the excited state population in a molecule using coherent control will be given.

P55 **Ceramide modulates raft properties in monolayers and bilayers**

Jesse J. Popov, Ira, Linda J. Johnston (*National Research Council Canada, Steacie Institute for Molecular Sciences*)

Ceramide is a sphingolipid that has been implicated in diverse cellular functions ranging from differentiation to apoptosis. It is produced in plasma membrane rafts by enzymatic hydrolysis of the phosphocholine headgroup of sphingomyelin. Once introduced into the membrane, the unique physical properties of ceramide are responsible for altering raft characteristics. Such changes play critical roles in cell function, particularly in cell signaling where ceramide causes the coalescence of rafts into larger signaling platforms. In order to directly observe such effects, we have systematically incorporated ceramide into model monolayers and bilayers that mimic the composition of native membranes. This was done either by premixing lipid solutions containing ceramide substituted for various equimolar amounts of sphingomyelin, or by producing ceramide *in situ* by adding sphingomyelinase. Taking advantage of the high resolution of atomic force microscopy, we observed in all cases that ceramide caused distinct changes in membrane properties. In monolayers, these modulations depended on the mole fraction of ceramide as well as on the surface pressure. In bilayers, ceramide caused changes in condensed-phase domain size and topography, depending on the proportion of cholesterol in the system and on the method of ceramide incorporation. The diverse nature of the modifications witnessed in these systems, particularly in the liquid-ordered phase which parallels raft composition, suggests directly that the properties of ceramide alone may account for the innumerable cell functions under its influence. More generally, these findings emphasize that lipid properties themselves may be sufficient to drive plasma membrane processes, a consideration of substantial biological significance.

P56 Structural and functional dynamics in cardiomyocytes revealed by second harmonic and third harmonic generation, and multiphoton excitation fluorescence microscopy

Catherine Greenhalgh¹, Virginijus Barzda¹, Steve Elmore², Johannes HGM van Beek², Juerg Aus der Au³, Jeff Squier³ (*1-University of Toronto 2-Vrije Universiteit Amsterdam 3-Colorado School of Mines*)

Structural dynamics in cardiomyocytes have been investigated with simultaneous second harmonic generation (SHG), third harmonic generation (THG) and multiphoton excitation fluorescence (MPF) microscope. Mitochondrial physiological activity has been observed via intensity fluctuations (flickering) of THG signal. Random flickering as well as wave like propagation of THG intensity increase across the cardiomyocyte has been revealed. Similar observations can be seen in MPF, although the effect is less easily observable due to fluorescence photobleaching. Flickering dynamics showed sensitivity to uncouplers and inhibitors of electron transfer that links the physiological activity in mitochondria with fluctuations of THG efficiency. Simultaneously detected SHG signals were efficiently generated in the anisotropic bands of sarcomeres. Time series of 2-D SHG images revealed nanocontractions of sarcomeres. The length fluctuations (~ 150 nm per sarcomere) took place on the hundred millisecond time scale. The nanocontractions of different sarcomeres on the same myofibril were not synchronized and did not produce macrocontractions on the range of the whole cell. The interactions between the nanocontractions and the mitochondrial activity are also being investigated. The simultaneous detection SHG, THG and MPF microscopy appears to be a powerful tool for dynamic investigations of interactions between different organelles within a cell.

P57 Volumetric Characterization of Conformational States of Apomyoglobin

Nicolas Taulier and Tigran V. Chalikian (*Leslie Dan Faculty of Pharmacy, University of Toronto*)

At room temperature, apomyoglobin may exist in its native, molten globule, or unfolded state depending on the solution pH and ionic strength. Specifically, at neutral pH, the protein is in its native state. At pH 4, it exists in the molten globule conformation. At pH 2, apomyoglobin is unfolded at low salt but adopts the molten globule conformation when [NaCl] exceeds 200 mM. We have employed high precision densimetric and ultrasonic velocimetric techniques to measure changes in the relative specific sound velocity increment, $\Delta[U]$, partial specific volume, Δv , and partial specific adiabatic compressibility, Δk_s , accompanying acid- and salt-induced native-to-unfolded, native-to-molten globule, and molten globule-to-unfolded transitions of apomyoglobin at 25°C. We interpret our measured volumetric parameters in terms of the hydration properties and interior packing of each of the conformational states of apomyoglobin. In general, our data shed light on the molecular forces that stabilize/destabilize the native and denatured states of globular proteins.