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The University of Pennsylvannia was funded by Air Force Office of Scientific Research to establish and continue eight projects in science and engineering research. Complete reports were presented for three projects in the Technical Report for September 30, 1994 through September 29, 1995. Four additional reports were presented in the Technical Report for September 30, 1995 through September 1996. This document presents the final reports for all eight funded projects: (1) Studies of High Performance Electrooptic Polymers and Devices, (2) Molecular Modeling, (3) Research Programs in Elementary Particle Physics and Astrophysics, (4) High Speed Network, (5) Cognitive Science (6) NMR Spectroscopy of Membrance Proteins (7) Gene Expression and Protein Chemistry (8) Biotechnology of Cell-Cell and Virus Cell Interactions

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SCIENCE AND ENGINEERING PROGRAMS AT THE UNIVERSITY OF PENNSYLVANIA

Grant No. F49620-94-1-0453
FINAL TECHNICAL REPORT
SEPTEMBER 30, 1994 to SEPTEMBER 30, 1999

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EXECUTIVE SUMMARY

The University of Pennsylvania was funded by the Air Force Office of Scientific Research to establish and continue eight projects in science and engineering research. Complete reports were presented for three projects in the Technical Report for September 30, 1994 through September 29, 1995. Four additional reports were presented in the Technical Report for September 30, 1995 through September 29, 1996. This document presents the final reports for all eight funded projects:

- Studies of High Performance Electrooptic Polymers and Devices. A new class of electrooptic chromophores with high thermal stability have been developed and studied.

- Molecular Modeling. (1) Real time quantum dynamics of lithium para-hydrogen clusters has been investigated. (2) Complex molecules and their interactions with their environment, such as surface or interface, have been studied.

- Research Programs in Elementary Particle Physics and Astrophysics. (1) Department of Physics and Astronomy laboratory space has been renovated for research programs in Elementary Particle Physics and Astrophysics. (2) A new program in Experimental Cosmology has been initiated.

- High Speed Network. All 178 data outlets in the Graduate Research Wing of the Moore School (Engineering and Applied Science) were rewired using category 5 twisted pair wiring. In addition, several improvements were made to the Ethernet backbone. All of the 10base2 network hubs have been replaced with 10baseT switches using category 5 twisted pair wiring. This replacement program was also initiated for 3 Engineering Departments the Towne Building.

- Cognitive Science. Thirteen thousand square feet of contiguous research space has been developed to house the Language, Logic and Computation, and Perception and Action programs of the Institute for Research in Cognitive Science.

- NMR Spectroscopy of Membrane Proteins. Three and four-dimensional NMR experiments have been implemented on peptide and protein samples in both solid and solution states. These experiments are a major advance toward the overall goal of protein structure determination.

- Gene Expression and Protein Chemistry. The new phosphoimager has been used extensively to study gene expression, notably in embryonic cells and in polymorphonuclear leukocytes challenged with chemotactic agents.

- Biotechnology of Cell-Cell and Virus-Cell Interactions. Novel technologies to understand the mechanics and specificity of biological adhesion to surfaces have been developed. These technologies have been applied to an understanding of cell-cell, virus-cell, and DNA-DNA interactions.

STUDIES OF HIGH PERFORMANCE ELECTROOPTIC POLYMERS AND DEVICES

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Molecular Design of High Temperature Nonlinear Optical Chromophores

Nonlinear optical processes in π-electron organic and polymer systems have attracted considerable interest because their understanding has led not only to compelling technological promise but also to new phenomena, new theoretical insights, and new materials and devices. The π-electron excitations occurring on the individual molecular, or polymer chain, units are the basic origin of the observed nonresonant nonlinear optical coefficients that are often unusually large. The coefficients are broad band and ultrafast, and, as shown by theory and experiment, their sign, magnitude and frequency dependence are determined by many-body electron correlation effects. This level of understanding, in turn, now makes viable molecular computer aided design of new nonlinear optical chromophores.

At the same time, as the field has naturally progressed toward technological applications, the main issues have focused on high performance materials that comply with device manufacturing and end-use conditions. New challenges in materials synthesis are being presented, calling for new methods and materials. Recently, new high performance optical grade polyimides have been developed to act both as passive waveguides and as hosts for EO chromophores in active regions of devices. However, oftentimes the EO chromophores cannot withstand the stringent thermal and chemical stability conditions required in polyimide-based fabrication processes and device assembly steps. In this report, we will discuss the thermal stability properties of a newly designed class of EO chromophores and their second order optical properties.

New High Thermal Stability EO Chromophores

We have successfully developed a new SY class of high thermal stability (T>365°C) fused-ring EO chromophores shown in Figure 1 that were designed using molecular computer aided design (CAD) prior to actual materials synthesis. The new EO chromophores were purposely designed to share structural features and materials properties similar to polyimide repeat units.[1] The donor-acceptor groups are selected to yield large EO coefficients and improved thermal stability as well as solubility in polyamic acid solutions.

The thermal stabilities of the pure SY chromophores have been studied by TGA (thermal gravimetric analysis) and DSC (differential scanning calorimetry) methods. The TGA data for the pure chromophores are shown in Figure 2. In reference to the parent SY156, the thermal stability increases upon ring substitution with donor groups of increased strength. The strong amine donor substituted SY215 is thermally stable up to 400°C. The measured linear absorption spectrum of SY215 is shown in Figure 3 inset.

Thermal stability studies were also conducted for the SY series dissolved in new high temperature optically transparent polyimides (Hitachi OPI series). The guest-host polyimide thin films were prepared by spin-coating, followed by soft vacuum baking and thermal curing. The films were heated to an elevated temperature in a nitrogen purged oven, and then cooled down to room temperature for measurements of their linear absorption spectra. In the important case of SY215/OPI-2005, for example, there are no spectral changes for the guest-host system up to 325°C.

Second Order Optical Properties of EO Chromophores

The molecular second order optical properties of SY215 were measured using DCSHG. The probe beam was supplied by a tunable source based on optical parametric generation and amplification in KTP crystals. [3] DCSHG measurements were made at seven wavelengths in the near infrared region ranging from 1600 nm to 1960 nm. The measured values of $\mu_x\ \beta_x(-2\omega;\omega,\omega) + 5kT\langle\gamma(-2\omega;\omega,\omega,0)\rangle$ are shown in Fig.3 along with the direct comparison to the theoretical results for SY215. Both the theoretical and experimental results, which are fairly well matched in the entire region, show the dispersion resulting from the 2ω resonance due to the $S_0 \rightarrow S_1$ transition at 1.86 eV, where S_0 denotes the ground state and S_1 the first excited state. The value of β_x can be estimated by subtracting the calculated third order contribution (23%) from the total, resulting in a value of 268×10^{-30} esu for $\beta_x(-2\omega;\omega,\omega)$ at $h\omega$=0.65 eV.

The theoretical results reveal the origin of the large second order optical response. The largest contribution to $\beta_{xxx}(-2\omega;\omega,\omega)$, the dominant component of $\beta_x(-2\omega;\omega,\omega)$, arises from the virtual excitation sequence $S_0 \rightarrow S_1 \rightarrow S_1 \rightarrow S_0$. The charge separated nature of the first excited state results in both a large dipole moment difference and a large transition moment between S_0 and S_1 states. The contour diagram of the difference in the charge density distribution between the S_0 and S_1 states shown in Fig. 1(b) clearly illustrates the transfer of π electrons from the donor substituted imidazole ring to the naphthoylene moiety, and consequent large charge separation. The contour diagram of the transition density matrix between S_0 and S_1 exhibits similar features. Contributions due to virtual excitation sequences involving higher lying excited states not considered in the standard two-level model decreased the value of $\beta_{xxx}(-2\omega;\omega,\omega)$ by 27%.

In summary, SY215 possesses high thermal stability (T≈400°C) and a large nonresonant second order optical susceptibility (268×10^{-30} esu at 0.65 eV), which is larger, for example, than that of the standard chromophore dimethylamino-nitrostilbene (DANS). SY215 illustrates how a new EO chromophore class can be designed and actually realize high performance EO polymer materials that comply with optoelectronic integrated device manufacturing and end-use conditions.

Acknowledgments

We gratefully acknowledge generous support from the Air Force Office of Scientific Research, Advanced Research Projects Agency, and Pittsburgh Supercomputing Center.

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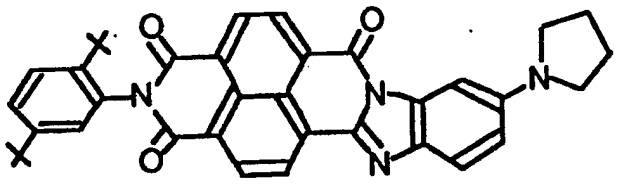

Fig. 1. Schematic molecular structure of 1,8-naphthoylene-(3'-pyrrolidino) benzimidazole -4,5-dicarbox-N-(2,5-di-tert-butyl) phenylimide (SY215).

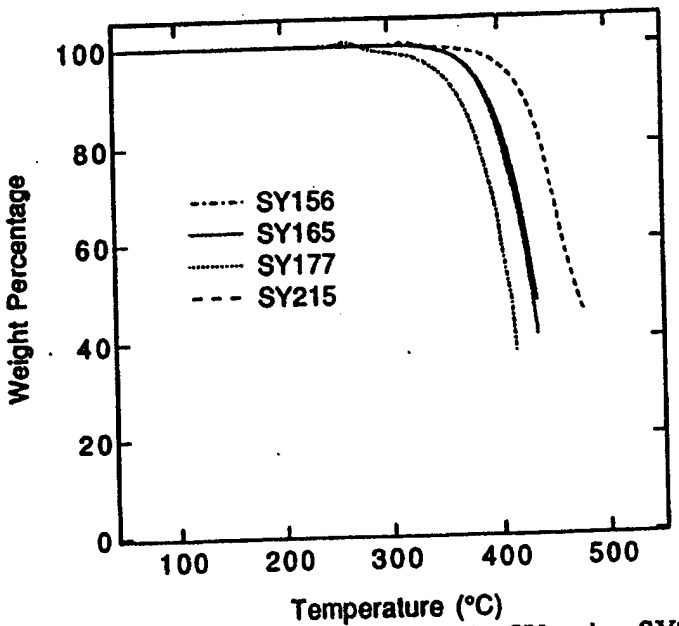

Fig. 2. Thermal gravimetric analysis (TGA) results for the SY series. SY215 exhibits less than 5% of weight loss near 400°C.

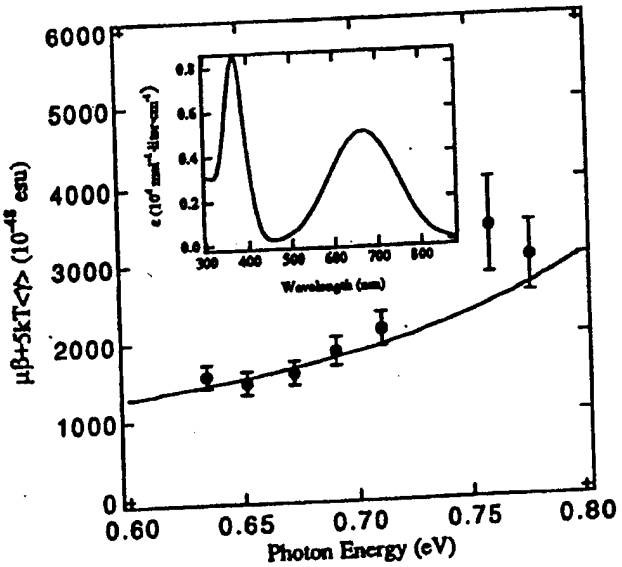

Fig. 3. Experimental (points) and theoretical (solid line) values for SY215 of μ_x $\beta_x(-2\omega;\omega,\omega)$ $+\langle\gamma(-2\omega;\omega,\omega,0)\rangle > 5kT$ as a function of photon energy. μ_x is measured to be 4.4 D. And room temperature T is 300 K. Inset: linear absorption spectrum of SY215.

MOLECULAR MODELING

K. Kinugawa, Visiting Scientist
P.B. Moore, Postdoctoral Fellow
M.L. Klein, Professor of Chemistry

Centroid Path Integral Molecular Dynamics Simulation of Lithium Para-Hydrogen Clusters. The real-time quantum dynamics of a series of lithium para-hydrogen clusters, $Li(p\text{-}H_2)_n$ (n=13, 55, and 180), at 2.5 and 4.0 K have been investigated by means of normal mode centroid path integral molecular dynamics (NMCMD) simulations. The equations of motion and the methodology of the NMCMD simulation, were based on the Nose-Hoover chain constant-temperature MD scheme and the reference system propagator algorithm (RESPA). In addition to the energetic and structural properties, the real-time semi-classical dynamics of the centroids of the Li atom and p-H_2 molecules in the clusters was explored to investigate the diffusive and vibrational properties associated with cluster melting. The self-diffusion coefficient of p-H_2 molecules in the $Li(p\text{-}H_2)_{13}$ cluster, which is completely melted at both 2.5 and 4.0 K, is comparable to the experimental value of bulk liquid p-H_2 just above the melting temperature. The melting of the $Li(p\text{-}H_2)_{55}$ cluster is incomplete at these temperatures though slow diffusion is occurring accompanied with spatial localization. The $Li(p\text{-}H_2)_{180}$ cluster exhibits the tendency of surface melting at 4.0 K while the core region remains rather solid-like. In general quantization of the nuclei, and the decrease of the cluster size, both enhance the ease of melting and diffusion, but the Li atom moves on the cluster surface nearly as freely as the surface p-H_2 molecules. Quantization causes the power spectra of the cluster velocity autocorrelation functions to be shifted to lower frequency than in the classical regime.

Quantum Dynamics of Lithium Ions at and near Surfaces. The properties of lithium atoms adsorbed on the surface of solid para-hydrogen, and also implanted in the near-surface sublayers, was investigated at several temperatures (2.5, 4.0, and 6.0 Kelvin) by means of computer simulations using NMCMD methodology. In this way, the approximate real-time quantum dynamics of a series of these systems is calculated following the scheme originally proposed by Cao and Voth [J. Chem. Phys. 101,6168 (1994)] and subsequently modified [Kinugawa, Moore, Klein, J. Chem. Phys. (1997)].

Specifically, the real time semi-classical dynamics of the centroids of the Li atom and para-hydrogen molecules are being explored. The energetic, structural, and diffusive properties of Li on and below the <111> surface of solid para-hydrogen are being investigated. The doped Li is initially being placed in four locations: on the surface, as well as in the first, second, and third layers below the surface. These simulations follow the diffusion of the Li from the lower layers to the surface, and finally evaporation off the surface. Preliminary results suggest that a vacancy in the surface is needed for Li diffusion to the surface and that the quantum nature of the Li does not seem to play a large role in this process. However, the quantum nature of hydrogen is important in creating the initial defect for the Li to diffuse into. Other surface defects and steps on the Para-hydrogen are being considered for investigation.

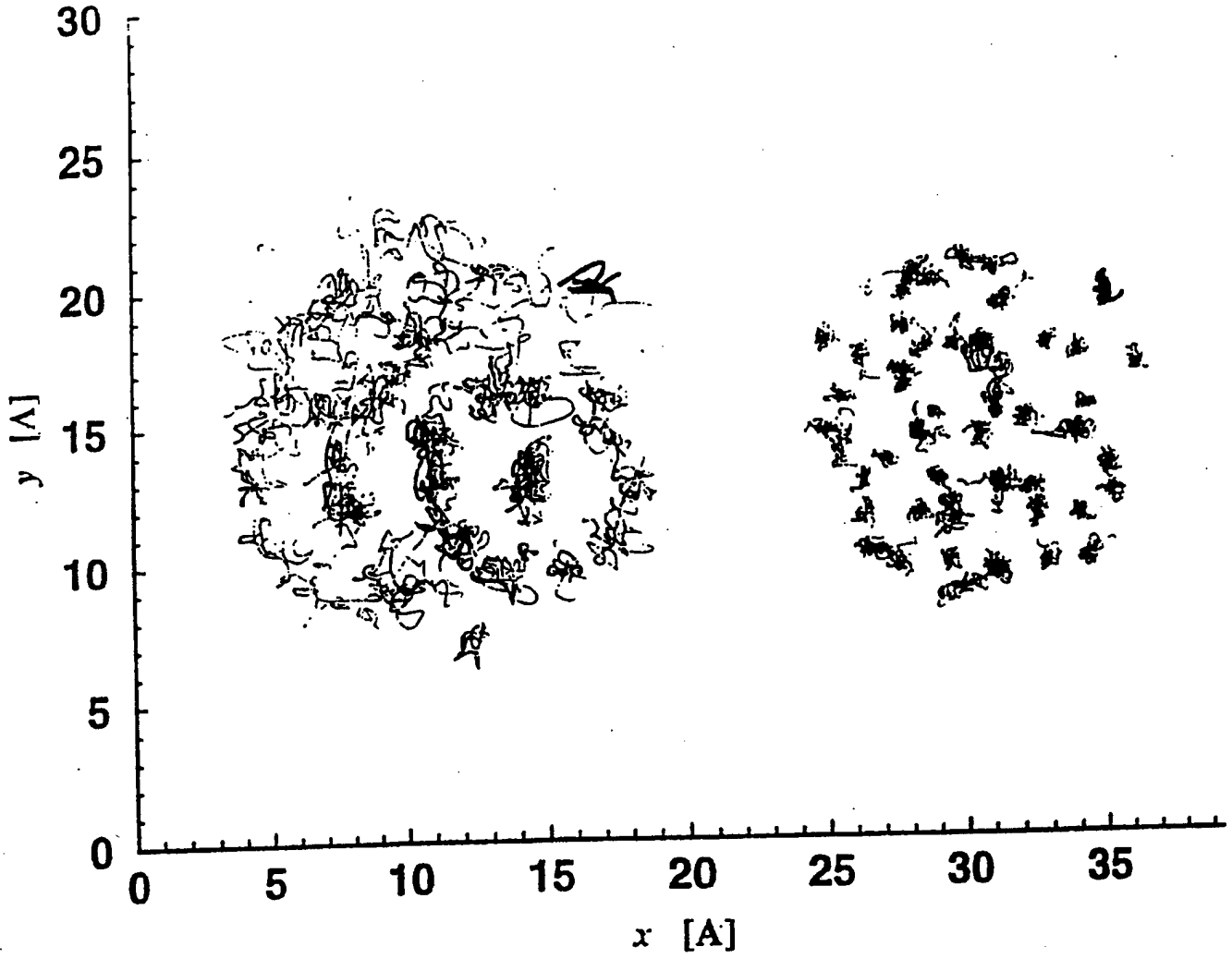

Figure 1: The xy-projection of the trajectories of the $Li(H_2)_{55}$ cluster covering 10 ps at T = 4K. Dots refer to H2 and the bold line to Li. The centroid trajectories are on the left and the classical trajectories on the right.

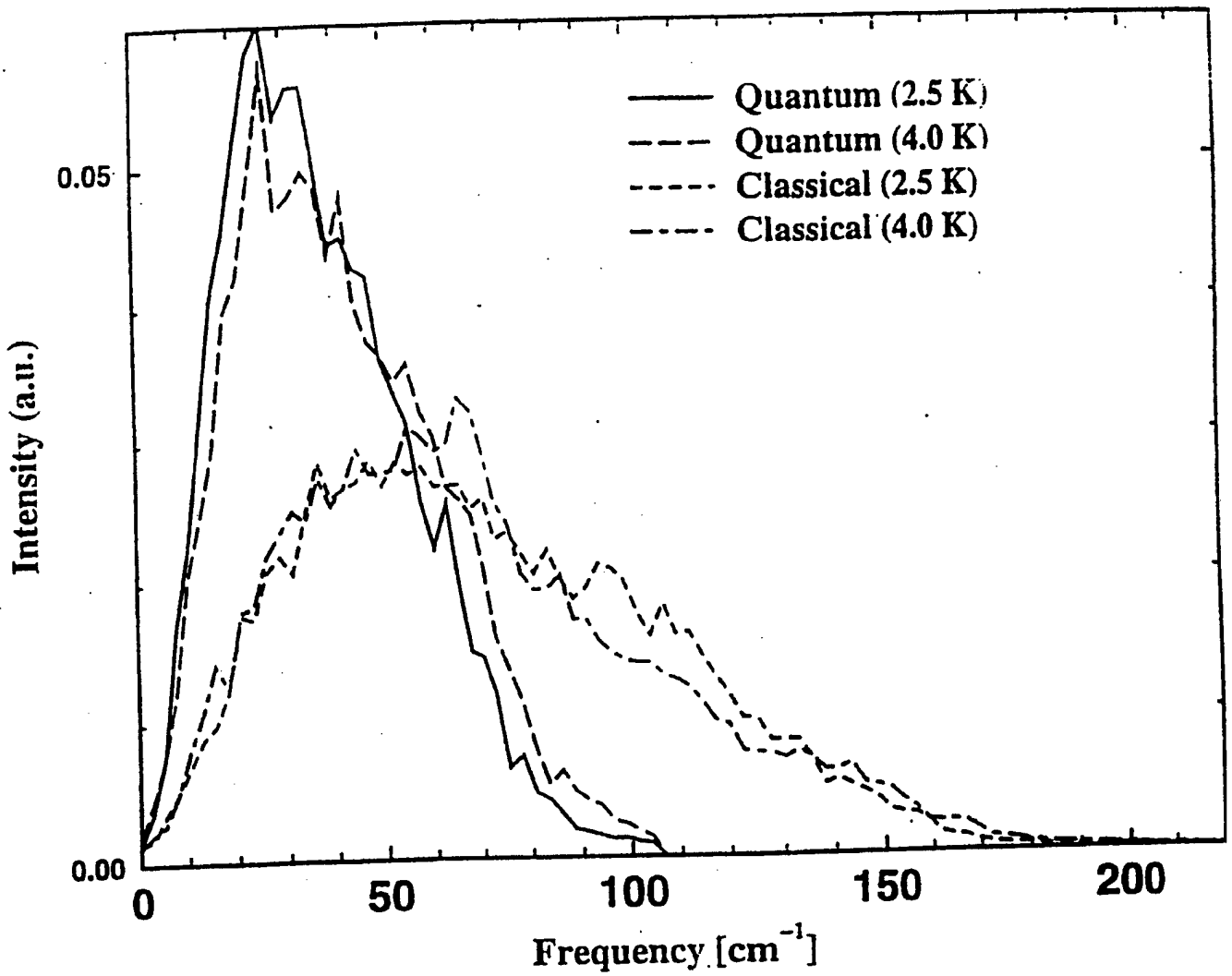

Figure 2: Normalized power spectra of the quantum velocity autocorrelation functions of H_2 in the $Li(H_2)_{55}$ cluster. The classical results are also plotted together for comparison.

RESEARCH PROGRAMS IN ELEMENTARY PARTICLE PHYSICS AND ASTROPHYSICS

Eugene W. Beier, Professor of Physics
Larry Gladney, Associate Professor of Physics
Robert J. Hollebeek, Professor of Physics
Nigel Stuart Lockyer, Associate Professor of Physics
Hugh H. Williams, Professor of Physics

Elementary Particle Physics and Astrophysics. This project consists of two components. The first consists of renovation of laboratory space in the Department of Physics and Astronomy for research programs in elementary particle physics and astrophysics. A related proposal was funded by the Academic Research Infrastructure program at the National Science Foundation and the renovations proceeded.

Experimental Cosmology. The second component of this project consists of the New Initiative in Experimental Cosmology. Penn has been successful in establishing an outstanding research group in experimental and theoretical cosmology. Since 1994 three outstanding young investigators have accepted appointments to the faculty of the Department of Physics and Astronomy. These are Dr. Steven Myers, who joined Penn from the California Institute of Technology on October 1, 1995, Dr. Chung-Pei Ma, who also came from the California Institute of Technology and arrived July 1, 1996, and Dr. Mark Devlin, who came to Penn from Princeton at the beginning of the 1996-1997 academic year.

The research program undertaken by these individuals consists of theoretical and observational work related to the early universe. The experimental work is directly related to the Cosmic Microwave Background radiation, which will be studied with both the techniques of ground-based radio astronomy (Myers) and balloon-borne microwave observations (Devlin). Dr. Ma carries out large scale numerical simulations of the observational consequences today of various assumptions about the specific composition of the very early universe.

Principal expenditure in support of astrophysics research was to support the work of Myers. The focus of Myers' research at Penn is to determine the fundamental cosmological parameters and to explore the fossil record of the early universe as regards the process of galaxy and star formation. Myers has used primarily the tools of radio astronomy to work towards these ends – centimeter and millimeter-wave interferometry and radiometry. In particular, he has conducted a series of observational programs to detect anisotropy in the microwave background, to measure the Sunyaev-Zeldovich effect from clusters of galaxies, to find a large number of new gravitational lens systems suitable for cosmological studies, and to study the powerhouses at the centers of radio galaxies and quasars. Myers has also studied the theoretical issues relevant to the use of these observations to measure quantities of cosmological interest. Significant projects include the CLASS gravitational lens survey, and the Cosmic Background Imager (CBI).

The study of anisotropy in the cosmic microwave background radiation has moved to the forefront of observational cosmology, due to the successes of a number of experiments. Much of the interesting cosmological information is found on angular scales less than a half degree and will be in accessible to the planned satellite missions. Myers has been involved in conducting some of the pioneering microwave background observations on angular scales from 2 to 7 arcminutes using the radio telescopes of the Owens Valley Radio Observatory. He and collaborators at the California Institute of Technology made a series of observations measuring cosmic background fluctuations on scales from 7 to 22 arcminutes. He prepared a paper detailing observations of the scattering of the microwave background light by ultra-hot gas that surrounds massive clusters of galaxies, the so-called Sunyaev-Zeldovich effect. These radio measurements have been combined with X-ray satellite measurements of emission from the same clusters to yield an estimate for the Hubble constant, the parameter that determines the scale and expansion rate of the Universe. To further investigate the microwave background anisotropies, Myers has been involved in the design and construction of a new instrument: the Cosmic Background Imager (CBI). The novel interferometric design of the CBI will allow it to map and measure CMB fluctuations on the arcminute to 20 arcminute scales, complementing the larger-scale measurements of the MIDEX mission and balloon experiments. With a graduate student, Myers is leading the program to map the SZ effect in nearby clusters using the CBI.

His other major project is the Cosmic Lens All-Sky Survey (CLASS), a multi-institutional international collaboration to map over 10,000 radio sources in order to discover new gravitational lenses suitable for cosmology, especially the determination of the Hubble constant and to place limits on the cosmological constant through lens statistics. Myers led the first phases of the survey, which began in 1994. Seventy-five hundred sources have been mapped, from which new lenses have been discovered. This largest single radio lens survey in the world is in the forefront of gravitational lens studies.

HIGH SPEED NETWORK PROGRAM

Ira Winston
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School of Arts and Sciences; School of Engineering and Applied Science

Graduate Research Wing (GRW) of the Moore School. GRW houses a significant portion of the Computer and Information Science and Electrical Engineering departments as well as the Computing and Educational Technology Service group that supports the entire School of Engineering and Applied Science. Existing data wiring was unsuitable for use with current data networking products. All of the data outlets in the GRW were rewired using category 5 twisted pair wiring.

The 10base2 network hubs were replaced with 10baseT hubs and 10baseT switches.

The University of Pennsylvania was one of the initial thirteen non-supercomputer sires to be awarded connections to the NSF-sponsored vBNS network. The introduction of fast Ethernet (100baseT) and additional ATM connections permit ATM connection to the vBNS network. The new wiring in GRW allows the vBNS network to be extended at full speed to any location in the building.

Moore School. The Moore School was wired with category 3 twisted pair wiring and RG58 coaxial cable. In the first phase of this network upgrade, 10baseT hubs were installed and the migration from 10base2 to 10baseT using the category 3 twisted pair wiring was started. In the next stage, 10baseT Ethernet switches were installed and the building rewired with category 5 twisted pair wiring to allow for higher speed networking. The Moore School is occupied by faculty members in Computer and Information Science and Electrical Engineering.

Towne Building. The Towne Building was wired with category 3 twisted pair wiring and RG58 coaxial cable. . In the first phase of this network upgrade, 10baseT hubs were installed and the migration from 10base2 to 10baseT using the category 3 twisted pair wiring was started. In the next stage, the Towne Building was rewired with category 5 twisted pair wiring to allow for higher speed networking. The Mechanical Engineering and Applied Mechanics, Bioengineering, Systems and Chemical Engineering departments are housed in the Towne Building.

Kalpana Etherswitch. A fifteen port Kalpana ethernet switch was purchased to replace the shared bus Ethernet backbone. The switch connects wiring closets throughout the School of Engineering and Applied Science as well as the four primary mail/file/timesharing servers. Several thick Ethernet backbone cable that connected wiring closets were replaced with fiber optic Ethernet cables connected to the Kalpana etherswitch. There has been an observable improvement in both network performance and reliability as a result of this upgrade. The upgrade benefits all of the faculty research described in the original proposal.

In the last phase, additional rewiring was done in the School of Engineering and Applied Science and additional Ethernet switches were installed connected to one another using ATM/fast Ethernet.

COGNITIVE SCIENCE

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Professor, Computer and Cognitive Science
Co-Director, Institute for Research in Cognitive Science

Lila R. Gleitman
Professor, Psychology
Co-Director, Institute for Research in Cognitive Science

Renovation of the Institute for Research in Cognitive Science is now complete. Twelve offices, 2 laboratories, and 3 conference rooms were created, adding 6,500 square feet to this research area and bringing together a total of 13,000 square feet of contiguous research space. The total cost was $1,100,000. The Institute is concerned with the study of how humans and machines process information, both visual and linguistic, and manipulate environment. Besides scientific research, the Institute is involved in technology transfer with several major industries such as GE, IBM, Unisys, Boeing, GM, AT&T, and Bellcore. In addition, Penn is the site for NSF's National Center for Cognitive Science. This Center had an initial award from NSF in 1991 for over $8,000,000 for five years. The Center has recently been awarded a six-year renewal for $12,000,000. This renewal and the expanded scope of the Institute's program necessitated the identification and renovation of this additional space.

The Institute for Research in Cognitive Science pursues a long range research agenda with three scientific foci: Language Acquisition, Structure, and Processing; Logic and Computation; and Perception and Action. Some specific projects in these foci are:

- Language: acquisition of the verb lexicon and syntax, use of prosodic information, computational models, and integration of some of these in the context of animated agents; grammar formalisms and integration of syntax, semantics, and discourse functions; integration of statistical and structural information; experimental studies of human sentence comprehension; and, role of discourse context in this resolution.

- Logic and Computation: models of resource bounded computation, modal logic, and conversational plans; proof theory and grammatical theories; and, representation and logic of partial information.

- Perception and Action: perception of organized visual space, and accurate motion through it; execution of gross and small motor programs; signal to symbol to signal transformation; high-level vision and cognition; and, perception of language processing.

Special emphasis will be given to the development and consolidation of research in cognitive neuroscience. The Institute develops programs for undergraduate and graduate education, outreach programs with public schools and cultural institutions emphasizing science education, and technology transfer activities including collaborative projects and partnerships with industry.

This research is having a direct impact on such technologies as robotic planning and manipulation, the integration of information from different sensory modalities, graphics and animation, spoken and written natural language interfaces, mechanical translation, information retrieval systems, software development, and bioinformatics. Some of these developments will have a major impact on ensuring widespread access to digital libraries, on advanced manufacturing technology, and on the efficient use of health-care resources. Developments in the technologies for information processing provide a foundation for the efficient delivery of critical services. These services will be the decisive determinants of success in the competitive global marketplace and in the maintenance of national security in the years ahead. It is clear, therefore, that Cognitive Science will play a central role in maintaining and strengthening the nation's scientific and technological leadership in the coming century. This renovation will allow the continuation of these very successful research efforts.

NMR SPECTROSCOPY OF MEMBRANE PROTEINS

S.J. Opella, Professor of Chemistry

The overall goals of the research program are to develop and apply NMR spectroscopy for the study of proteins. Substantial progress has been made during the past year, and both aspects of the program have benefited from the support.

Further development of NMR methods and instrumentation is important because of the limitations of current methods of structural biology, including x-ray crystallography and multidimensional NMR spectroscopy, especially with regard to structure determination of membrane proteins. A general approach is being developed for the study of membrane proteins that utilizes complementary information for solution NMR studies of micelle samples, solid-state NMR studies of bilayer samples, and molecular dynamics simulations. The principal area of spectroscopic development has been the approach to protein structure determination for oriented samples. In the past year, a family of three- and four-dimensional solid-state NMR experiments have been implemented on single- and poly-crystalline peptide samples and then extended to proteins in phospholipid bilayers. These experiments give high resolution along the three frequency axes from the 1H chemical shift, 1H-15N heteronuclear dipolar, and 15N chemical shift interactions, enabling uniformly 15N labeled protein samples to be studied. This is a substantial advance because it means that the samples can be prepared by expression in bacteria where uniform labeling is inexpensive and facile. These spectra yield direct measurements of the three orientationally dependent frequencies that are needed to determine the orientation, and hence the structure, of each peptide plane in the protein. Once resolution and measurement of spectral parameters are accomplished, then assignment of the resonances to specific residues in the protein becomes an important issue. Here four-dimensional variants of the three-dimensional correlation experiments that incorporate either dilute- or abundant- spin exchange have been developed and show considerable promise as systematic assignment schemes.

In order to apply these solid-state NMR methods, as well as complementary solution NMR methods, it is necessary to prepare relatively large amounts of pure proteins labeled with stable isotopes, such as 2H, 13C, and 15N. During the past year we have made considerable progress in the expression and purification of membrane proteins in bacteria where there is the greatest flexibility for isotopic labeling. Samples of Vpu, virus protein "u" from HIV-1, merT, the mercury transport protein of the bacterial mercury detoxification system, and the M2 channel peptides of the acetylcholine receptor and the NMDA receptor have been prepared for the first time during the past year. This has opened up the possibilities for structure determination of these important biological systems.

The development and implementation of solution NMR spectroscopy of these membrane proteins in micelles has continued. The availability for the first time of uniformly 15N labeled samples of these proteins gives opportunities to use the most powerful three- and four-dimensional solution NMR experiments to determine their structures on the basis of NOEs, J coupling constants, and chemical shifts. The availability of the 750 MHz solution NMR spectrometer has been particularly valuable for these studies. The high quality of the data indicate that it will be possible to compare the structures of these membrane proteins determined by solid-state NMR in lipid bilayers and solution NMR in micelles. It also means that it will be possible to characterize the dynamics of these proteins over a broad range of timescales, which will give insight into their functions and organizations.

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GENE EXPRESSION AND PROTEIN CHEMISTRY

A. Cashmore, Professor of Biology
J. Ecker, Associate Professor of Biology
R. Schultz, Professor of Biology
S. Zigmond, Professor of Biology

Anthony Cashmore
Most of our original goals concering to the use of the phosphorimager are being met. We have published several papers describing our work.

Joseph Ecker
The MD Phosphorimager has been used for the successful analysis of over 500 images.

Richard Schultz
We have used the phosphorimager on a routine basis to examine the regulation of gene expression in the embryo following fertilization and during the maternal-to-zygotic transition. In addition, we used the imager to examine the ZP2 to $ZP2_f$ change that accompanies fertilization. In this regard, we have used the imager for the three stated objectives. We have also used the imager for analysis of Northern blots and gel shift assays. Although these were not stated objectives at the time, our research needs have evolved to these very suitable applications for this technology.

Interactions and collaborations:

Annual Meeting of the Teratology Society, San Juan, Puerto Rico (1994)
14th Annual Ottawa Reproductive Biology Workshop, Ottawa, Canada (1995)
Annual Meeting of the Society for the Study of Reproduction, Davis, CA (1995)
Gordon Conference on Fertilization and Activation of Development, New Hampshire (1995)
The Basic Research and Clinically Applied Technology of *In Vitro* Fertilization, Boston, MA (1995)

Department of Zoology, The Charles University, Prague, The Czech Republic (1994)
Department of Animal Science, University of Missouri, Columbia, MO (1995)
The Jackson Laboratory, Bar Harbor, ME (1995)

Sally Zigmond
In response to a number of chemotactic agents, polymorphonuclear leukocytes (PMNs) undergo an actin-based change in their cytoskeleton that is involved in the chemotactic response. During the past year we have been able to simplify the functional assay of the G protein signal transduction cascade from that using cells permeabilized with Streptolysin-O to one using cell lysates. These lysates now increase their F-actin level when stimulated with GTPγS. Since the open nature of the lysate makes it much more ameable to study, we have been characterizing this system and defining the componets necessary for the F-actin increase. For this reason, we have postponed the study of protein phosphorylation of particular actin binding proteins. These studies remain part of our future plans.

Degrees Awarded:

Patrick Dunn, Ph.D. - (1989-95) Toward a Yeast Artificial Chromosome-based Genome Map of Arabidopsis. Ph.D. Awarded May 95

Gregg Roman, Ph.D. - (1989-95) Genetic Analysis of Ethylene Signal Transduction and the Positional Cloning of the EIN2 Locus. Ph. D. awarded May 95

Ann Lehman, Ph.D. - (1989-85) Molecular and Genetic Characterization of the Hookless Mutants in Arabidopsis thaliana. Ph.D. Awarded December 1995

Publications:

Ahmad, M. and Cashmore, A. R. (1995). A blue-light photoreceptor requires phytochrome A or phytochrome B to mediate blue-light responses in *Arabidopsis thaliana*. submitted. :

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Lehman, A., Black, R., and Ecker, J.R. HOOKLESS1, an ethylene response gene, is required for differential cell elongation in the Arabidopsis hypocotyl. submitted for publication.

Lin, C., Ahmad, M. and Cashmore, A. R. (1995). Arabidopsis cryptochrome 1 is a soluble protein mediating blue-light-dependent regulation of plant growth and development. submitted. :

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Lin, C., Ahmad, M., Gordon, D. and Cashmore, A. R. (1995). Expression of an Arabidopsis cryptochrome gene in transgenic tobacco results in hypersensitivity to blue, UV-A, and green light. Proc. Natl. Acad. Sci. USA. **92** : 8423-8427.

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Terzaghi, W. B., Bertekap, R. L. and Cashmore, A. R. (1995). Members of the GBF family of bZIP transcription factors are principally localized in the cytoplasm in cultured Arabidopsis and soybean cells. submitted. :

Terzaghi, W. B. and Cashmore, A. R. (1995). Light regulation of transcription. Annual reviews of plant physiology and plant molecular biology. **46** : 445-474.

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Worrad, D.M., Ram, P.T., and Schultz, R.M. (1994). Regulation of gene expression in the mouse oocyte and early preimplantation embryo: Developmental changes in Sp1 and TATA box-binding protein, TBP. *Development* **120**, 2347-2357.

Worrad, D.M., Turner, B.M., and Schultz, R.M. (1995). Temporally restricted spatial localization of acetylated isoforms of histone H4 and RNA polymerase II in the 2-cell mouse embryo. *Development* **121**, 2949-2959.

BIOTECHNOLOGY OF CELL-CELL AND VIRUS-CELL INTERACTIONS

Daniel A. Hammer, Professor of Bioengineering

During the period of this award, the Hammer laboratory developed a number of novel technologies to understand the mechanics and specificity of biological adhesion to surfaces. These technologies have been applied to an understanding of cell-cell, virus-cell, and DNA-DNA interactions.

Cell-cell adhesion. Biological cells, such as leukocytes, adhere to surfaces via receptor-mediated interactions. We have been interested in a particular class of receptors, called selectins, and their mechanism of action. Selectins bind to carbohydrates and mediate the rolling of leukocytes on blood vessel walls. Rolling is a dynamic friction, and our efforts have been directed at understanding how these molecules work to support this unique type of adhesion. Our major achievement was the development of a colloidal mimetic, bearing selectins or selectins ligands, which can recreate the adhesion of leukocytes. These "cell-free"systems, in which we can recreate rolling adhesion with any of the molecules of the selectin family, demonstrate that rolling is a function of the physical chemistry of selectin adhesion molecules, and not due to cellular features such as roughness, deformability and signaling [1-4]. A particular accomplishment with the cell free system are that we can recreate the shear threshold effect – a phenomenon seen in cells and previously attributable to cell features such as microvilli – therefore, the shear threshold effect is really due to the physical chemistry of adhesion molecules [4, 5]. We further extended the construction of the "cell-free" systems to biodegradable microspheres that could be used for drug delivery or carrying imaging agents, most recently derivatizing poly-lactic glycolic acid microspheres to have the adhesive properties of leukocytes, but filling them with inflammatory agents [6].

Virus-cell interactions. We have been making progress on both simulations and experiments to understand the docking and entry of viruses into cells. We have developed a new technique, called Brownian Adhesive Dynamics, to simulate the adhesion of viruses to cell surfaces via receptors, and are applying it to the binding of HIV particles to the cell surface. We identified that the equivalent site hypothesis, which often used to make an estimate of the rate and strength of viral binding, is significantly in error. The improved algorithm will be used to assess the binding of different strains of HIV to host cells. A paper on this work is currently in preparation. Experimentally, we have been working with Dr. Robert Doms in Microbiology at Penn to measure the fusion of cell lines expressing different forms of HIV surface molecules using a micropipette aspiration assay. In this assay, two cell lines bearing complementary fusion molecules are brought into contact using pipettes, and the fusion or coalescence is monitored on the stage of a microscope. Fluorescent dyes are used to monitor the mixing of membrane and contents between cell types. The purpose of this assay is to assess the complementarity of different potential fusogenic molecules of different strains of HIV.

DNA-DNA interactions. We have been using microarray technology and bead adhesion to assess whether bioadhesion can be used to detect unknown strands of DNA. In the assay, micro scale

beads are derivatized with a single chain oliogonucleotide using biotin-avidin chemistry (oligos are derivatized at the 5' end with a biotin, and bound to avidin coated beads). Correspondingly, surfaces are micropatterned with different biotinylated DNA-oligonucleotide strands by attaching them to avidin-coated surfaces using an arrayer. When the DNA on the bead is complementary to the DNA on the surface, the beads bind avidly; if the DNA strands are not complementary, the beads do not bind. Then, we can assess how the strength of adhesion depends on the number of complementary DNA strands on the bead and the surface, the degree of overlap of the oligonucleotide strands, and the hydrodynamic force that acts on the particles. We have shown that a 20 base-pair overlap is sufficient to overcome the hydrodynamic force on a 10 micron diameter bead up to 1000 1/s shear rates. We are now assessing whether hydrodynamic forces can used to screen high fidelity DNA base overlaps from those with minor defects, such as single nucleotide defects. Ultimately, this technique may be used to identify a third unknown strand from a foreign microbe, such as a pathogen, by using an array of beads in solution, each with a strand that might be complementary to one end of the unknown strand, and a array of spots on the surface, each which is complementary to the other end of the unknown strand. Thus, the methodology may be used for optical detection of pathogenic organisms. A paper on this work is currently under development.

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