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12. Abstract (Maximum 200 words)
   This report on the Conference on Assembly and Self-Assembly at the Interface of Biology, Chemistry and Physics held in II Ciocco, Italy on August 20-25, 200, includes an overview, the program, attendee list and presentation abstracts.
   Recent advances, such as scanning probe microscopy, genetic approaches in molecular biology, optical traps, and single molecule microscopy and spectroscopy, created a natural interface between the worlds of chemistry, physics, and biology by exploring the nanometer scale. This is the fundamental length where novel mesoscopic materials confront the building blocks of life, proteins and DNA. These developments in nanoscience, together with visions of nanotechnology and lessons from cell and molecular biology define a current challenge: Manufacturing complex, heterogeneous structures of well-controlled architecture and function. The attainment of this objective requires a deeper knowledge of assembly and self-assembly in biology, chemistry, and physics. More specifically, the design of fundamentally new materials needs an understanding how the desired structural theme or complex function, is "coded" into the molecular architecture of the reactants. The study of assembly and self-assembly is essential to all three disciplines. However, the design parameters, the control strategies and the nature of the end products differ widely in these fields. The proposed interdisciplinary conference aims to promote exchange of ideas between different and weakly interacting communities, namely physics and chemistry of soft matter, supramolecular chemistry, and cell and molecular biology. The conference offered the sessions on cutting edge research in assembly and self-assembly:

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Grantee Report on the
Euroconference on "Assembly and Self Assembly at the Interface of Biology,
Chemistry and Physics", Il-Ciocco, Italy, August 2001
supported by ONRIFO Grant number N00014-01-1-1009

Recent advances, such as scanning probe microscopy, genetic approaches in
molecular biology, optical traps, and single molecule microscopy and spectroscopy,
created a natural interface between the worlds of chemistry, physics, and biology by
exploring the nanometer scale. This is the fundamental length scale where novel
mesoscopic materials confront the building blocks of life, proteins and DNA. These
developments in nanoscience, together with visions of nanoengineering and lessons
from cell and molecular biology define a current scientific and technological challenge:
Manufacturing complex, heterogeneous nanostructures of well-controlled architecture
and function.

The attainment of this objective requires a deeper knowledge of assembly and
self-assembly (ASA) as encountered in biology, chemistry, and physics. More
specifically, the design of fundamentally new materials depends on an understanding
how the desired structural theme or complex function, is "coded" into the molecular
architecture of the reactants. The study of ASA is already well developed in each of the
three disciplines. Yet, the design parameters, the control strategies and the nature of the
end products differ widely in these domains. The benefits of closer interactions between
the three different disciplines are widely recognized. Such contacts are however
hampered by differences in nomenclature and by paucity of interdisciplinary forums.

The ASA interdisciplinary conference was designed to promote exchange of
ideas between different and weakly interacting communities concerned with the
challenge defined above. In particular, it aimed to bring together leading scientists active
in physics and chemistry of soft matter, supramolecular chemistry, and cell and
molecular biology. Furthermore, it attempted to enhance contacts between industrial and
academic research laboratories. To this it brought together a group of experts committed
to such an interdisciplinary exchange with a focus on the well-defined themes of
assembly and self-assembly. It intentionally avoided topics such as protein folding and
membrane physics where interdisciplinary research is already well established.

It is convenient to describe the motivation leading to organization of the
conference from the perspective of soft matter physics. In this community the study of
ASA focused on two primary topics. One is the self-assembly of amphiphilic molecules, soaps, lipids and block copolymers, in the bulk and at interfaces. Within this domain one can further distinguish between two directions: The physics of membranes and monolayers formed by insoluble lipids and the physics of self-assembly of soluble soaps into equilibrium structures. The second topic is protein folding where the challenge is to understand the statistical physics underlying the relationship between the chemical sequence of a protein and its unique three-dimensional structure in the folded state. Both topics reached a level of maturity evident in the publication of treatises and major review articles. The fields described above focused on an important yet narrow set of systems: amphiphilic molecules and heteropolymers. Furthermore, the systems studied were characterized by the existence of equilibrium or a constrained equilibrium state. The scope of the ASA field is much wider as illustrated by examples from biology, supramolecular chemistry and industry. A case to the point is the assembly of viruses. Simple viruses, such as the Tobacco Mosaic Virus undergo thermodynamically controlled self-assembly of the capsid proteins on a template provided by the viral RNA. The assembly of more sophisticated viruses such as the T4 bacteriophage is no longer thermodynamically controlled. Rather, it relies on temporal control of a number of assembly pathways. An example of assembly involving very different systems and length scales may be drawn from the oil industry. It is sometimes necessary to block underground fractured rock formations within the reservoir in order to enhance the efficiency and the environmental acceptance of water sweeps. One technological solution involves selective formation of gels within the rock fractures. This form of large-scale assembly involves control of the flow pattern of the reaction mixture as well as temporal control of the gelation. These two extreme examples illustrate two points: One is that the field encompasses a wealth of systems that were not considered traditionally within the "ASA community". A second and related point is that there are many diverse disciplines involved and there is a lack of forums allowing for communication between them.

At this juncture it is also important to note that the different disciplines tend to adopt distinctive approaches to the study of ASA problems. The soft matter community studies the fundamentals of assembly and self-assembly and their interpretation in terms of statistical mechanics. Supramolecular chemistry is concerned with the manipulation of directed interactions in order to design structures. Finally, molecular biology relies heavily on the manipulation of the genetic apparatus to gain insights on biological
assembly pathways. It is hoped that the ASA conference will promote cross-fertilization between the different communities. An illustrative example of a problem requiring an interdisciplinary approach concerns the entry of plasmid DNA into a living cell where it functions as an active gene. The molecular mechanism involved is not yet established. The elucidation of this mechanism is likely to require methods and ideas from molecular biology as well as from soft matter physics and polymer science.

The discussion presented above suggests that it is desirable to establish an extended ASA community that will encompass a wider spectrum of disciplines and approaches and will deal with a broader selection of ASA problems. The program of the ASA conference was constructed with this aim in mind. The choice of topics intentionally aimed for subjects that are “off the beaten track” of interdisciplinary research. The ASA theme and the “off the beaten track” criterion were supplemented by two additional guidelines. One is to favor systems that are, broadly speaking, “biologically inspired”. The second is to attempt to combine presentations originating from different and weakly interacting communities. To ensure the interdisciplinary nature of the conference we made an effort to maintain a balance between the different disciplines and communities represented among the participants.

Interdisciplinary conferences are not free of risk. The primary danger is that members of the different communities will fail to interact and communicate. We have taken a number of steps in order to minimize this risk. First we have chosen invited speakers that are committed to the idea of seeking collaborations and interactions outside their own field and capable of giving talks to a mixed audience. Second, the ASA theme of the conference is sufficiently focused so as to allow practical discussions of future collaborations. Finally, the conference venue imposed a modicum of isolation. This, together with the duration of the conference encouraged interactions.

The adopted conference format was similar to that of Gordon conferences in the sense that all talks were invited and allocated equal time slots. The program was designed so as to give a series of interlocking talks covering cutting edge research in the ASA field. In particular, the program offered the following sessions:

- The DNA session exemplified a problem of self-assembly in its full complexity ranging from the polyelectrolyte nature of DNA to the membrane-DNA complexes formed during the transfection of cell with a gene.
• The session on the **Extracellular Matrix and the Cytoskeleton** showed how polymers assemble into smart micro-machines – a problem, which needs the synergy between soft matter physics and cell biology to be fully understood.

• Nanofabrication is currently a main focus in solid state physics and colloid chemistry. Our session of **Virus Assembly** illustrated nature’s ability to form perfect nanovessels and the routes afforded by supramolecular chemistry to create architectures reminiscent of viruses.

• **Biominalization** is a good example of how inert and living matter can interface. The speakers in this session represented fundamental research as well as successful efforts to use insights gained from the study of biominalization to couple proteins to semiconductors.

• Protein folding is now a well-established field. In the session on **Hierarchical Self-Assembly** we grouped closely related but less recognized topics, among them RNA folding and hierarchical self-assembly in synthetic model systems.

• While the ancient Chinese have discovered silk, the scientific basis for its exceptional properties remains an open question. The **Silk Road** session was concerned with three aspects of this problem: the biology of spider silk, biomimetic synthesis of silk-like materials, and the production of high performance synthetic fibers.

• Biological systems often operate far from thermodynamic equilibrium. As Alan Turing realized this allows the self-assembly of patterns in reaction diffusion systems. The session on **Nonlinear Pattern Formation in Cell Biology** was devoted to this topic focusing on pattern formation by amoebae.

• The wiring of neurons and the way to interface neuronal assemblies with semiconductors pose a major challenge. The session on **Assemblies of Neurons** aimed to present this topic within the general framework of assembly and self-assembly.

• Exciting insights concerning ASA were obtained from novel **Nanomanipulation Techniques**. The leading experimental techniques will be discussed in the session.

• ASA are already widely used in technological applications. An **Industrial Perspective** involving the paint and oil-recovery industries was presented in this session.
A discussion panel on the Future Technological Impact of ASA with particular emphasis on biomimetic materials and the role of interdisciplinary research.

In organization the conference we also attempted to promote interactions between academia and industry. The program offered a special session devoted to Industrial perspective on ASA research and applications. Speakers from a number of industrial laboratories participated in other sessions. In addition, a number of Industrial companies that contributed to the funding of the conference sent representatives to attend the conference.

More details concerning the conference can be found in the accompanying the book of abstracts and the Addendum. The following changes should be noted. R. Goldstein and A. Belcher were unable to attend. The information reached us at the last moment thus preventing us from finding suitable substitutes. A. Bausch replaced D. Weitz. Altogether, the conference comprised of 35 invited talks, a panel discussion focusing on the role of interdisciplinary research in developing ASA based technologies and a poster session. While all talks were of high quality, the talks by Isralachvili, de Gennes, Gerisch, Engel, Molineeux, Cunnigham, Knight, Evans, Pincus, Florin, Stossel, Stoddart, Fromhertz, Ringsdorf, O'Brien and Chateney were especially notable in both content and style. The conference was attended by a total of 109 participants.

It is gratifying to note that the opinions of the participants, during the conference and immediately afterwards, were overwhelmingly positive. The choice of topics and the quality of the speakers were described in highly complementary terms. The feedback also suggested that the conference succeeded in promoting interdisciplinary contacts. The main criticism voiced concerned the density of the program and the relative lack of free time.
Dear Sir

Enclosed please find six copies of the proceedings of the EuroConference on “Assembly and Self-Assembly at the Interface between Biology Chemistry and Physics” that was held in II Ciocco, Italy, in August of 2001 with the support of ONR grant N00014-01-1-1009. Also enclosed is a report describing the Conference.

This is an opportunity to express, yet again, our appreciation for the generous support afforded by ONRFO as well as its efficient handling of our proposal.

Sincerely

A. Halperin

CEA-Grenoble, DRFMC-SI3M, 17 rue des Martyrs, 38054 Grenoble Cedex 9, France.
# Addendum, Update and Corrections

## Schedule:

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<td>8:15am-10:15am</td>
<td>Pattern Formation (start 9:15am)</td>
<td>Extracellular Matrix: Cytoskeleton Stossel, <strong>Bausch</strong></td>
<td>DNA: Gene Therapy Saffiya, Cunningham</td>
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<td>Biomineralization Mann, <strong>Boucher</strong> Special Session: de Gennes</td>
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Schedule Changes:

Monday, August 20th:
Opening Session
P.-G. de Gennes talk moved to special session Thursday, Aug. 23rd 11:30 am.

Tuesday, August 21st:
Nonlinear Pattern Formation in Cell Biology
11:30am speaker change
R. Goldstein No Longer Attending. Session will end at 11:30am

Wednesday, August 22nd:
The Extracellular Matrix and the Cytoskeleton – From Model Systems to Reality: The Cytoskeleton
9:15am speaker change
D. Weitz replaced by

“Probing Local Properties in Complex Biomaterials”
A. Bausch, Harvard University; Cambridge, USA
Abstract:
Recent advances in biology have resulted in an accumulation of information with an unprecedented complexity, suggesting the need for a fundamental understanding of the underlying mechanisms. An excellent example for the complexity is the dynamic and localized response of the cytoskeleton, which is a cytoplasmic system of polymeric structures. Here we describe the development of new physical techniques and model systems to address the complexity of these collective interactions of the many cytoplasmic constituents, which are critical for many cellular processes such as mechanical stability, cell motility, adhesion and intracellular transport processes.

Multi-particle tracking of colloidal probes is used to study the local properties of actin networks, a model system for the cytoskeleton. Transport processes in such networks were characterized.

In cellular systems, magnetic colloidal probes were used to quantify the local viscoelastic properties. We observed mechanical heterogeneity in the cytoplasm. It was shown that measurements of viscoelastic properties enable real-time study of the contraction of endothelial cells, yielding important insights into the biochemical regulation.

Thursday, August 23rd:
Biomineralization
11:30am speaker change: A. Belcher No Longer Attending
Replaced by special session with P.-G. de Gennes.

Friday, August 24th:
2:00pm (14:00) schedule re-arrangement

2:00pm-3:00pm - E. Evans
3:00pm-4:00pm - D. Leckband
4:15pm-5:15pm - E.-L. Florin
5:15pm-6:15pm - F. Brochard-Wyart (As Scheduled)
Poster Additions:

"Gating of Ionic Channels via Fluctuation-Mediated Interaction Between Membrane Inclusions"
1) Cheol-Min Ghim, 2) Jeong-Man Park
1) Seoul National University, 2) The Catholic University of Korea

"Probing Cell Surface Using Colloids"
Christophe Huber, Claire Arambel and Nelly Henry
Centre de Recherche Paul Pascal -CNRS

"Aggregation and Shape Transformations in W/O Microemulsions"
Forschungszentrum Juelich

"Characterisation of Nanosized Oxide Particles and Nanocomposite Coatings Prepared via Modified Emulsion Precipitation"
Forschungszentrum Juelich

Poster Correction:
Page 45:
"Biomimetics within the Air Force Research Laboratory"
Rajesh Naik, Sean Kirkpatrick, Lawrence Brott, Laura Sowards, Sharon Jones, Sarah Stringer and Morley Stone
United States Air Force Research Labs

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Welcome to the
Conference on Assembly and Self-Assembly
at the Interface of Biology, Chemistry and
Physics
Il Ciocco, Italy, August 20-25, 2001

Organizers: A. Halperin (SPrAM, Grenoble, France), J. Kas (University of Texas at
Austin, USA)

International Advisory Committee: P.G. de Gennes (France), J.N. Israelachvili
(USA), T. Pollard (USA), H. Ringsdorf (Germany)

Local Organizing Committee: A. Halperin (SPrAM, Grenoble, France), J. Kas, M.
Forstner, D. Martin (University of Texas at Austin, USA)

The conference is made possible by the gracious support from:

The European Union; The US Office of Naval Research; Office of Naval Research
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Shlumberger; CEA

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SELECTIONS AND INVITED SPEAKERS:

Opening Session

"Biomimetic Objects and Soft Actuators"
P.-G. de Gennes, ESPCI; Paris, France

Abstract:
Living beings often produce remarkable structures, such as the silica shells of diatoma, or the sophisticated structures of striated muscles. One major current problem is to invent useful objects inspired by these structures, but simpler, and able to be produced in short times. Some examples will be presented.
"Subtleties and Differences in the Interactions of Biological and Non-Biological Molecules and Surfaces"

J. Israelachvili, University of California at Santa Barbara; Santa Barbara, USA

Abstract:

Recent SFA, AFM, Optical Trapping, and other measurements of the interactions and forces between biological surfaces and molecules show that these forces can be much more complex than expected from simple two-body interaction theories, such as the DLVO theory. Biological interactions differ from classic colloidal interactions in many ways: a biological interaction is generally a ‘process’ involving a sequence of individual two-body interactions that progress in a well-orchestrated fashion in both space and time. Thus, a binding event at one place can have an effect or trigger another interaction somewhere else (spatial dependence), and non-equilibrium, rate-dependent and time effects often play a crucial role (temporal dependence). The elemental interactions that make up a biological process are typically a mixture of short-range and long-range forces, specific and non-specific, and each one depends on different factors. These different interactions and the factors that affect them will be reviewed, with examples given of how they combine in such biological processes as membrane adhesion and fusion, recognition interactions, and transport.

D. Leckband and J. Israelachvili “Intermolecular Forces in Biology” Quart. Revs Biophys. (in press)
Nonlinear Pattern Formation in Cell Biology

"Cell Biology and Nonlinear Dynamics"
E. Bodenschatz, Cornell University; Ithaca, USA

Abstract:
Many eukaryotic cells show a chemotactic response to spatio-temporal chemical gradients. Examples range from unicellular organisms to human cells involved in the immune system. In contrast to bacterial chemotaxis, eukaryotes do not need to move to sense a chemical gradient. The cell membrane is homogenously covered by receptors and cell sizes can be small (10 microns). The questions are: How do cells detect chemical gradients? What are the cellular processes that are needed for polarization?

One prototype system for cellular development and chemotaxis is the social amoeba Dictyostelium discoideum (dicty). It is believed that dicty has similar molecular networks for chemotaxis as other eukaryotic cells. Upon starvation, dicty turns on a sophisticated genetic program during which cells develop a chemical relay system involving the detection, production and release of cyclic AMP (cAMP). This relay process creates a pattern of macroscopic spiral waves, which chemotactically guide the cells towards aggregation centers. Subsequently cells differentiate, a migrating slug is formed, and this multicellular part of the life cycle ends with the development of a fruiting body.

Thus, chemotaxis to traveling concentration waves plays an indispensable role in dicty biology. Dicty is well suited for the study of this capability since a large number of cells can be developed simultaneously and reproducibly. In addition, a library of strains with GFP-fused proteins is available for the optical study of intracellular and extra cellular dynamics. It has been experimentally shown that the chemotactic response of dicty to a gradient of cAMP requires translocation of the cytosolic protein CRAC to the cell membrane. A release of cAMP from a pipette elicits a translocation of CRAC to the nearside of the cell. Later the cell extends pseudopods towards this direction and moves up the gradient.

In this talk, we will first review existing experimental results on dicty chemotaxis. Then, we will present a model that could explain the observed behavior, especially the very rapid response timescale. Finally, we will discuss planned experiments, which will both test this specific model and which will provide more a quantitative characterization of the decision-making steps in this process.

The work is conducted in collaboration between Cornell U. (I. Rafols, T. Tanaka, E.B.) and the University of California at San Diego (W.Rappel, P.Thomas, H. Levine, Bill Loomis). We gratefully acknowledge support by the NSF-Biocomplexity program.
"Intracellular Pattern Formation Based on the Actin System"
G. Gerisch, Max-Planck-Institute for Biochemistry; Munich, Germany

Abstract:
Amoeboid cells like those of Dictyostelium have no stable polarity, but in order to move persistently they have to polarize into a leading edge and a tail. This establishment of cellular organization occurs in a quasi-periodic fashion and is based on supramolecular structures formed by the cytoskeleton, primarily by the actin system. Actin exists in an equilibrium between monomeric G-actin and filamentous polymers (F-Actin). A large variety of actin-binding proteins determines this equilibrium and controls the assembly of actin filaments into higher-order structures. In fast moving cells like neutrophils or Dictyostelium cells the actin system is highly dynamic; reorganization occurs within a few seconds either spontaneously or in response to external signals. The role of actin-binding proteins in promoting this reorganization is being determined by physical methods under defined conditions in vitro as well as by genetic manipulation in the context of the living cell. The modeling of these data on single proteins into a network of macromolecular interactions will be a challenge for theoretical studies.

A highly sophisticated organization of the cytoskeleton is required for cell division, a process in which segregation of the chromosomes is coordinated in space and time with the formation of a cleavage furrow separating the daughter nuclei. Mutant cells of Dictyostelium lacking the conventional double-headed myosin II proved to be an excellent system to study patterning of the cell cortex into polar regions and a cleavage furrow. These myosin II-null cells are unable to divide in suspension, thus becoming multinucleate. When brought into contact with a solid surface, cells are even in the absence of myosin II capable of dividing by the formation of multiple cleavage furrows. This process is preceded by the sorting out of actin-binding proteins, in the same way as it occurs in normal cells undergoing bipartite division. By tagging relevant proteins with green fluorescent protein (GFP), this protein sorting can be recorded in vivo. Examples analyzed are coronin, a protein enriched at the polar regions of a dividing cell, and cortexillin, which accumulates in the cleavage furrow. Elimination of each of these proteins by targeted gene disruption has shown that both contribute to proper cell division.

"Dynamic Polymorphism in Bacterial Flagella and Bacterial Filaments"
R. E. Goldstein, University of Arizona, Tucson, USA

Abstract:
In this talk I discuss two related phenomena involving novel nonlinear dynamics and elasticity associated with self-assembled structures at the scale of bacteria. These are bacterial flagella and bacterial filaments. 1) During the run-and-tumble swimming of peritrichously flagellated bacteria such as E. Coli and Salmonella, the bundling and unbundling of the flagella are intimately linked to chirality transformations that propagate down the helices. A beautiful experiment by Hotani many years ago showed that such transitions can be induced periodically in detached flagella that are pinned to a microscope slide at one end and subjected to a steady fluid flow. I will discuss a theory for Hotani's experiments that quantitatively describes his observations. 2) Certain mutants of B. subtilis form long filamentary assemblages of cells when cell separation after division fails. As these filaments grow, they supercoil much like DNA, forming highly-organized plectonemes of macroscopic size. I will describe current attempts to understand the mechanism of this supercoiling based on nonequilibrium stresses induced in the cell wall by growth, and various aspects of low Reynolds number elastohydrodynamics that have been elucidated in the course of these studies.

DNA: From Electrostatics to Gene Therapy: Polyelectrolyte and Protein Induced Assembly

"Self-assembly and structure of neurofilaments"
P. Jannney, University of Pennsylvania; Philadelphia, USA

Abstract:
Intermediate filaments are composed of largely alpha-helical proteins that self-assemble into linear polymers with diameters on the order of 10 nm and lengths of several microns. These cytoskeletal filaments are considerably larger in diameter than are actin filaments, but they are also much more flexible, with typical persistence length on the order of a few hundred nm. Among different classes of intermediate filaments, neurofilaments are particularly interesting because they contain in addition to their highly anionic filament core, a series of long extended polypeptide chains with alternating positive and negative charged regions that protrude from the filament surface and are thought to mediate interactions with other filaments or with membranes. We present a series of studies by light and atomic force microscopy, dynamic light and neutron scattering, and viscoelasticity characterization to relate the microscopic structure of these filaments and their electrostatic characteristics to their macroscopic network and bundle formation. In particular we describe the strong effect of multivalent counterions on filament interactions that can lead to both formation and redissolution of neurofilament complexes.


"Intelligent Micro- and Nanocapsules"
H. Moehwald, Max Planck Institute of Colloids and Interfaces; Golm, Germany

Abstract:
In recent years much has been learnt to prepare molecular surfaces and films in a defined way. This knowledge has now been transformed to coat colloids which could then be destroyed to obtain hollow capsules. Coating these again with lipid double layers simple cell models are formed. Their wall permeation can be controlled via pH, ionic strength and thermal treatment and they can be loaded by drugs or enzymes. In rather simple experiments the mechanical properties of these artificial cells could be determined and meanwhile also the outer surface could be functionalized for specific recognition. This yields new possibilities for biophysics as well as applications in encapsulation and release.

G. Decher: Science 277 (1997) 1232
Virus Assembly

"Phage Virions and Genome Translocation Into the Host Cell"
I. Molineux, University of Texas at Austin; Austin, USA

Abstract:
The proteinaceous head or capsid of many double-stranded DNA phage particles is only about 2 nm thick. In the lambdoid phage HK97 and the Pseudomonas phage D3 the capsid is strengthened by cross-links between protein monomers but in most phages that have been examined the capsid can be completely disrupted into monomer capsid protein by heat and detergents or other denaturing agents. The mature capsid is permeable to small ions and in some instances DNA intercalating dyes can be dialyzed into the virion. However, the polyamines in T4 particles are resistant to removal, their concentration inside the virion reflects those of the host cell in which the phage particle was made. Nevertheless, polyamines are not essential and T4 growth occurs in cells that do not contain them.

The DNA molecule in double-stranded DNA phage particles is estimated to be at a concentration of ca. 500 mg/ml, approximately the same concentration found in DNA condensed by the presence of polyamines or polyethylene glycol. In isometric icosahedral heads the DNA is wound around an axis that is colinear with the tail, in prolate heads the axis of DNA coiling is perpendicular to the axis defined by the tail. Packaged DNA appears to be in B-form with an inter-duplex spacing of about 2.4 nm. Despite the DNA concentration and the thin capsid shell purified virions are remarkably stable, usually retaining full infectivity for many months.

The concept that double-stranded DNA phages act like a syringe stemmed from the classic experiment of Hershey and Chase. This thought subsequently led to the supposition that DNA is packaged under pressure in a phage particle and that ejection of DNA would be spontaneous once the particle was "unplugged". However, these widely held ideas have never received any direct experimental support. To the contrary, experimental evidence shows that some phages cannot follow the syringe-pressure model. The rate of transfer of phage SP82G DNA into Bacillus subtilis has been shown to follow Arrhenius kinetics, and penetration of the infected cell by naked T5 DNA occurs normally after the capsid is removed.

My laboratory has developed an assay that measures the kinetics of phage T7 DNA internalization by the cell. T7 DNA translocation is also different from that of most other phage types in that transcription of the phage genome is necessary. If transcription is blocked then only ~850 bp of the phage genome efficiently enters the cell. Long-term incubation of the cell-phage complex allows more T7 DNA to be internalized but only by what appears to be a stochastic process. Phage mutants have been isolated where an altered internal structural protein allows complete T7 genome entry in the absence of transcription. The kinetics of genome entry can be fitted to an Arrhenius plot and DNA translocation is interpreted as being enzyme-catalyzed. The necessary energy is supplied by the proton motive force.

"Supramolecular Polymers"
J. F. Stoddart, University of California at Los Angeles; Los Angeles, USA

Abstract:

TOPOCS TO BE COVERED

- Rotaxanes
- Catenanes
- Daisy Chain Polymers
- Pyridyl Crown Ethers
- Self-Assembly
- Molecular Recognition
- Template-Directed Synthesis
- Thermodynamic Control
- Multivalency

References:
“Toward Interlocked Molecules Beyond Catenanes and Rotaxanes” Org. Lett. 2000, 2, 2947-2950
"Nonviral Vectors? Polymer Therapeutics for Lysosomal and Endosomal Drug Delivery"
H. Ringsdorf, Johannes Gutenberg University; Mainz, Germany
The Extracellular Matrix and the Cytoskeleton – From Model Systems to Reality: The Cytoskeleton

"Actin Organization and Remodeling in Cell Shape and Cell Migration: Coping with Complexity"
T. Stossel, Harvard Medical School; Boston, USA

Abstract:
Three-dimensional actin filament arrays determine many cell shapes. To change shape or move, cells remodel these structures. Adhesion receptors provide traction for cell crawling and cohesion to resist and sense forces in tissues. These receptors are firmly rooted in the sub-membrane actin cytoskeleton. Diverse chemical and mechanical perturbations acting on the cell surface lead to actin remodeling and motoring of myosins on actin filaments that cooperate to determine specific cell shape changes and locomotion. Actin assembly and disassembly both can produce membrane protrusion. Conversely, actin remodeling changes the expression, affinity and avidity of adhesion molecules. Signal transduction cascades mediate this bi-directional information flow. The signaling chemicals also localize to the actin cytoskeleton and respond to its architectural rearrangements. Such feedback monitoring is essential for cells to reshape their membranes outwards in one place, inwards at another, and for the ins and outs to have numerous configurations in form. Cells use a relatively simple toolkit of reactions to remodel actin. These reactions are: 1) nucleation of sequestered actin subunits; 2) filament elongation in the fast-growing (“barbed”) direction; 3) termination of elongation by barbed end capping; 4) acceleration of pointed end depolymerization; 5) filament severing; 6) filament branching; and 7) filament crosslinking (to each other and to membrane receptors and other cytoskeletal polymers). But complexity arises from scores of actin-binding proteins that run the reactions in response to upstream signals. The overlapping mechanisms of actin remodeling are like the seeming chaos of the free market that leads to a surprising degree of spontaneous order, preferable to the comforting simplifying dictates of imperial planning. A few limiting principles are interesting, but it is the details of individual specific reactions governed by particular components, researched by individual investigators, that inexorably enlighten us how cells invade and identify potential molecular targets against diseases.

References
“Actin Vesicles as Cell Models”
A. Bausch, Harvard University; Cambridge, USA
"Thermal Ratchets? Force Generation by Self-Assembly of Cytoskeletal Polymers"
M. Dogterom, FOM Institute AMOLF; Amsterdam, Netherlands

Abstract:
Forces generated by the self-assembly of actin filaments and microtubules play a role in cellular processes such as cellular locomotion and the motion of chromosomes during cell division. We have developed experimental techniques to study the forces that are generated by single growing microtubules in vitro. The intrinsic effect of force on the growth velocity and the so-called "catastrophe" rate of microtubules can be quantified in these experiments. Theoretical predictions based on Thermal Ratchet models will be compared to the experimental results.
"Cell Motility and Non-Brownian Polymer Dynamics"
D. Humphrey, University of Texas at Austin; Austin, TX

Abstract:
The actin-specific molecular motor, myosin II, plays a crucial role for mechanical stability and structure of actin networks. Our results unexpectedly show a mechanism how myosin II can fluidize actin networks. Using fluorescence microscopy of myosin and individual F-actin in vitro experiments show that small minifilaments of myosin connect actin and push the filaments along each other in suspension under ATP conditions. With increasing myosin-to-actin filament ratio the filaments assemble to a network of bundles, to a pattern of asters, and finally to compact clusters. Although self-assembly of higher ordered structures does not require ATP, the viscoelastic properties of an isotropic network containing actin and myosin is dependent on the nucleotide added. Under ADP conditions myosin behaves like a crosslinker, which in turn increases the gel-like behavior of the semi-flexible polymer network. However, when ATP is added, the myosin fluidizes the network. To determine the mechanism, we utilize a unique rheometer mounted on the microscope to observe the sliding motion of individual actin filaments and to simultaneously measure the macroscopic viscoelastic properties. Our experiments indicate that myosin II not only plays a role in the self-assembly of actin networks to higher ordered structures, it also drastically alters the viscoelastic properties of solutions.
Hierarchical Self-Assembly

"The Activity of a Single Helicase on a Single DNA Molecule"
D. Bensimon, Ecole Normale Superieure; Paris, France

Abstract:
New techniques allow for the manipulation of single molecules, DNA or proteins and for the monitoring of their interactions. After a brief review of the various existing manipulation techniques, I will describe the use of a magnetic trap to monitor in real time the unzipping of a single DNA molecule by a single helicase. The rate of opening, processivity and interaction time can be measured as a function of force and ATP concentration. Moreover the step size can be estimated from the spectrum of unzipping signal.
"On the Signatures of Intrachain Self-Assembly"
A. Halperin, CEA-Grenoble, Grenoble, France

Abstract:
Polymer elasticity, as described in the standard textbooks, involves forces, f, that increase monotonically with the end to end distance, R. On the other hand, force measurements on biopolymers reveal force laws exhibiting novel features among them plateaus. Plateaus can occur when the monomers of the chain exist in two interconverting states. Within this scenario, the plateaus are the signatures of a cooperative, one-dimensional coexistence i.e., when the domains along the chain incorporate many monomers. In marked distinction, the “standard” discussions of polymer elasticity focus on polymers whose monomers exist in a single state. Modeling the elastic behavior of “two state” polymers is of interest in order to extract molecular information from the force laws. With this in mind we will examine a number of scenarios involving the deformation of “two state” polymers. Most of the talk will be devoted to the extension force laws of Polysoaps and homopolypeptides. In both cases, the two states arise from intrachain self-assembly. Polysoaps are flexible water-soluble polymers that incorporate, at intervals, covalently bound amphiphilic monomers. In this case it is helpful to distinguish monomers that form intrachain micelles from those that do not. In the case of homopolypeptides, it is necessary to distinguish between monomers that form a helical domain from those that are in a coil state. Depending on the conditions, the f vs. R diagrams may exhibit one or two plateaus. The plateaus may reflect tension induced “denaturation” of the intrachain self-assembly or tension induced self-assembly. Finally, we will briefly discuss the confinement behavior of “two state” polymers, where the novel features arise because the effective Flory interaction parameter of “two state” polymers can depend on the monomer concentration. As a result confinement may lead to phase transitions because of the associated increase in concentration and the concomitant change in the solvent quality.

References:
General Reference:

Elasticity of Polysoaps (O.V. Borisov and A. Halperin):
(1) On the Elasticity of Polysoaps: The Effects of Secondary Structure
Europhysics Letters 34, 657 (1996)
(2) Polysoaps: Extension and Compression
Macromolecules 30, 4432 (1997)
(3) Extending Polysoaps in the Presence of Free Amphiphiles:
(4) O. V. Borisov and A. Halperin
Deformation of Globular Polysoaps: Extension, Compression and Extensional Flow
The European Physical Journal B 9, 251 (1999)

Elasticity of Polypeptides (A. Buhot and A. Halperin)
(1) The Extension of Rod-Coil Multiblock Copolymers and the Effect of the Helix-Coil Transition
(2) On the Elasticity of Helicogenic Polypeptides
Biophysics Journal (submitted)
DNA: From Electrostatics to Gene Therapy: Gene Therapy

"DNA-Lipid Complexes and Interactions with Cells: Supramolecular Assembly and Gene Delivery"

C. Safinya, University of California at Santa Barbara; Santa Barbara, USA

Abstract:

There is now a surge of activity in developing nonviral cationic-based gene delivery systems for therapeutic applications [1], in part, because of their nonimmunogenicity and ease of production, but also because the single largest advantage of nonviral over viral methods for gene delivery is the potential of transferring extremely large pieces of DNA into cells. This was demonstrated when partial fractions of order 1 Mega base pairs of human artificial chromosome was recently transferred into cells using cationic lipids (CLs) as a carrier although extremely inefficiently [2]. We will describe recent work on the self-assembled structures of CL-DNA complexes by the quantitative techniques of synchrotron x-ray diffraction. Distinct structures have been discovered including, a multilamellar structure with alternating lipid bilayer and DNA monolayers [3], inverted hexagonal structure with DNA coated by cationic lipid monolayers and arranged on a two-dimensional lattice [4], and lamellar phases containing polypeptides and cytoskeletal filamentous actin [5]. Significantly, recent confocal optical imaging has revealed that the mechanisms of gene release from complexes in the cell cytoplasm are dependent on the nature of the self assemblies. We will also describe materials applications of these nano-structured systems. Supported by NIH and NSF.

References
“Applications of Gene Therapy in Oncology”
C. Cunningham, PRN Research, Inc.; Dallas, USA

Abstract:
In the last decades, medicine has shifted from primarily a study in physiology to one in cell and molecular biology. The consequent new understanding of the most basic antecedents of disease has revolutionized many specialties, and none more so than oncology. However, to develop a more targeted approach to anti-cancer therapy requires an understanding of why cancer cells do not respect the usual limitations on cell growth and target therapy in the first place. Under normal conditions, cell division occurs only in response to external signals that activate intracellular pathways leading to the initiation of mitosis. These signals are generally transient, and further growth ceases once they are absent. In addition, normal cells maintain an elaborate self-destruct machinery that can be activated by other signals in response to injury or previous number of divisions. The malignant state can then arise from mutations in the genes encoding for any of the above signals. Most commonly, these mutated genes (oncogenes) produce altered signaling molecules so that growth-inducing pathways become stuck in the “on” state. Methods for correcting the result of this inappropriate activation can be roughly divided into two classes: small molecule inhibitors of the actual abnormal proteins or manipulations of the mutated genetic machinery itself to suppress the production of the abnormal protein. Examples of the first class would include tyrosine kinase inhibitors, selective estrogen receptor modulators, and farnesyltransferase inhibitors. The second method, that of actually altering the production of the oncogene product, requires a more creative approach since it is the machinery of a very specific gene that needs to be suppressed. Perhaps the most highly developed is the technique of antisense technology. In this, constructed sequences of nucleic acid complementary to known regions of a particular RNA are introduced by systemic infusion. This technique has been applied to several targets and clinical trials performed with good activity in several tumor types. A parallel method is to manipulate the editing of primary RNA into mRNA by the introduction of specific ribozymes. This method has the advantage that one molecule of ribozyme can affect many molecules of RNA. Currently ribozyme technology is being tested against the Flt-1 VEGF receptor. Both of these methods are highly specific for a unique mRNA molecule and so are highly targeted.

Other gene therapy approaches seek to capitalize on the genetic defects of cancer cells in order to target cytotoxic therapy. For example, approximately 50% of human tumors demonstrate some defect in the tumor suppressor pathway, p53. Therefore, the introduction of intact p53 genes into the tumor cells should restore their ability to abort neoplastic transformation. Or, even more directly, replicating viruses normally inhibited by the p53 system can be delivered with the result that replication, and cytotoxicity, only occurs in the p53-defective tumor cells. Adenoviruses have been the preferred vector for these approaches but a variety of other vectors, including retroviruses and adenov-associated viruses have also been employed. ONYX-015 is one example of a selectively-replicating virus that has been successful in direct injection studies and is currently being explored in a systemic infusion setting.

Finally, new methods of delivering genes to both malignant and normal tissue are being developed. The most innovative of these may be the infusion of genetically modified bacteria that show marked preference for malignant tissue.

Biomineralization

"Biomineral-Inspired Approaches to Nanotectonics”
S. Mann, University of Bristol; Bristol, UK

Abstract:
Organized-matter chemistry is concerned with the synthesis, characterization and application of complex materials that exhibit order on length scales from the molecular to macroscopic. Recently, new strategies have been developed for the synthesis of organized inorganic nanostructures based on biomolecular templates, facilitated self-assembly of nanoparticle building blocks, and morphological transformations in complex fluids. A key aspect of this approach is the integration of organic self-organization and inorganic assembly such that hybrid materials are constructed by direct or synergistic patterning. This principle will be illustrated using several examples of our most recent work including the synthesis and assembly of silica nanostructures in tobacco mosaic virus liquid crystals, DNA-driven self assembly of gold nanorods, and the synthesis of linear chains of BaCrO₄ nanoparticles and nanofilament arrays in water-in-oil microemulsions.
“Sea Shells – A Model to Interface Inert Matter and Proteins”
A. M. Belcher, University of Texas at Austin; Austin, USA
Industrial Perspective

“Polymer-Clay Complexes and Self-Assembling Fluids for Improving Oil Recovery Efficiency”
G. Maitland, Schlumberger Cambridge Research; Cambridge, UK

Abstract:
Two major drivers for new technology in hydrocarbon recovery operations are lowering the costs of creating wells and increasing the recovery factors from reservoirs. This talk will describe two examples of smart fluid technology where molecular assembly and self-assembly are exploited to address these issues.

Probably the most important cause of well drilling problems is the instability caused in weak shale rock zones using water-based drilling fluids. This is a particular problem with shales containing a high smectite clay content, where chemical potential differences between the rock pore fluid in the highly compacted clay electrical double-layers and the drilling fluid give large driving forces for transport of water. This leads to clay swelling, weakening, erosion and failure with consequent problems of wellbore instability and sticking of the drilling assembly, which if unchecked can lead to major delays or even abandonment of the well. This swelling phenomenon can be controlled by low molecular weight hydrophobic-hydrophilic polymers which absorb on the clay surfaces and assemble into stabilizing mono- and bilayers. Molecular simulations and experimental techniques probing a range of length scales have been used to elucidate the mechanisms involved and optimize the molecular design of more effective shale inhibitors.

Most reservoirs only produce 30-40% of the hydrocarbon in place. One important process to stimulate a reservoir and enhance production involves creating hydraulic fractures. This requires the use of viscoelastic fluids to create the fractures and place small particles within them to keep the pathways open. Traditional fluids are based on biopolymers such as guar, but they have a number of shortcomings in blocking the fractures which prevent the full potential of the fracture pathways from being realized. Recently a new generation of fluids based on surfactants forming worm-like micelles have been developed. The talk will describe how their self-assembly and disassembly characteristics can be exploited to enhance hydrocarbon productivity, and how an understanding of molecular structure-bulk property relationships is enabling the design of more efficient high temperature, salt tolerant systems.
"Matrix and Financial Influences on the Structural Selection of Self Assembly Systems in Water-Borne Coating Applications"
J. E. Glass, North Dakota State University; Fargo, USA

Abstract:
This presentation will review the type of hydrophobe-modified, water-soluble polymers (HMWSs) used for improving the application performance of water-borne coatings (WBCs). The most recent review in this area was published last February (1). As the understanding of the technology in this area advanced, there have been symposia with the more pertinent data published in Advances in Chemistry series books by the ACS (2). There are four performance criteria that have driven the acceptance self-assembling. HMWSs in the WBC area:

1. Minimization of the extensional viscosity of thickened solutions and aqueous dispersions. In non-coating applications this is reflected in less mechanical degradation of the thickener. In coatings this relates to lower elasticity and less spatter in roll and better misting in spray applications;

2. Obtaining coating formulations with higher viscosities at high shear rates (>104s-1);

3. Achieving lower viscosities at low shear rates (<2 s-1) in coating formulations that have met the criterion in 2, particularly in formulations containing small particle lattices (<100 nm);

4. Stabilization of disperse phases in aqueous media that result in better applied coating properties, such as film gloss. W-S Ps of any type can be hydrophobically-modified.

There are three used in the coatings industry: H-M hydroxyethyl cellulose, H-M alkali-swollable emulsions, these two represent the HM of unmodified ethoxylated urethanes (HEURs). The former two are broad mixtures of materials where the extend of H-M of each chain varies and their placement is unknown. This is also true of commercial HEURs, but the latter compositions can be synthesized as true model compounds. It is the study of these models that provide significant understanding of the mechanism that HMWSs provide in the improved application performance of W-B Cs. The compositions of the three HMWSs used in coating applications will be described, along with their market segregation in different segments of the WBC industries. Based on the studies of model HEURs (3, Annable, Glass, Francois, Persson, Almgren, and all of their coworkers, some of the references listed below) the association of HEURs in aqueous solutions will be described. The influence of the narrow molecular weight, well-characterized HEURs will then be described in neat aqueous solutions, in the presence of different types of water soluble surfactants, and in the presence of aqueous dispersions of lattices and of pigments, the primary components in a coatings formulation. The ultimate goal: the influence of such components on the behavior of WBCs will then be addressed and interpreted based on the more fundamental information. The adsorption of there self-assembly model HEURs systems alone on lattices (4) (Russel, et. al.) and in competitive adsorption with surfactants (Glass, et.al. on the disperse phases in a coating will also be addressed. The complexity of adsorption in more complex fluid mixtures e.g., coalescing aids) by Hulden, et.al., will also be described. The presentation will then turn to more recent developments that examine the role of the diisocyanate structures that bond the polyester diols together and bind the hydrophobes to that chain, in telechelic terminal hydrophobe positioning in linear, branched and multiple-branched geometries and in various geometries that place the hydrophobes in pendant positions in various grouping sizes.

The Silk Road

“Self- and Directed-Assembly of Protein Liquid Crystals, a Paradigm for Biomimetics”
D. Knight, Oxford University; Oxford, UK

Abstract:
Some proteins and mixtures of proteins with other classes of biomacromolecules form lyotropic liquid crystals (LLCs) in Nature. They can self-assemble into a wide range of mesophases with very different packing arrangements and a range of remarkable physical and chemical properties. For example lamellar LLCs can be formed from a mixture of different proteins and phospholipids to give membranous organelles with a wide range of functionalities. A range of mesoscopic and hierarchical structures can be produced in LLC systems by a variety of factors including phase separation, host molecules and nematic escape. Some LLCs continue to flow (liquid LLCs) while others are lightly cross-linked into LLC elastomeric solids or heavily cross-linked into solid LLC resins. Liquid crystal elastomers can be readily aligned by rather small strain, magnetic or electrostatic fields and this class of LLC can exhibit other truly exotic properties. Some biological LLCs exhibit contractility such as actomyosin in muscles and the costas of certain protists while some viruses make nano-scale hypodermic needles out of LLC coat protein to inject nucleic acid into cells. Solvent in all LLC phases is contained within compartments with tightly defined size while solvent channels in some phases have defined orientation as well as gap size. In Nature, these compartments or channels can be filled with minerals or other molecules to make sophisticated composite materials including bone, insect cuticle and silks. LLCs are tuneable and can sometimes be switched from one mesophase to another under the influence of a wide range of factors including water concentration, temperature, pH and ionic composition. These remarkable properties give protein LLCs enormous biomimetic potential.
"Synthetic Analogs to Natural Silk"
J. O'Brien, DuPont Experimental Station; Wilmington, USA

Abstract:
The quest for a synthetic fiber with the aesthetic qualities of silk was a key objective of early synthetic fiber researchers and clearly impacted the development of commercially important products like rayon, acetate and nylon. In the 1940s and 50s extensive research was carried out to provide silk and wool like fibers from polymers based on poly (alpha amino acids). More recently researchers in academic and industrial laboratories around the world have sought to reproduce the uniquely strong and tough filaments that are spun by certain orb-weaving spiders. Although some technical success has been achieved in preparing high molecular weight polymers from reactive amino acid precursors, conventional synthetic methods have failed to provide the sequence and compositional specificity necessary to capture the structural and functional attributes of natural silk. In this talk the design and synthesis of genetically engineered polypeptides that exhibit architectural specificity similar to that of natural, evolutionary silk proteins will be discussed. Details of their fiber structure and a model describing their mechanical behavior will also be presented.

"High Performance Fibers: Perfecting Stress Transmitting Macroscopic Structures by Molecular Design"
D. Sikkema, Magellan Systems International; Arnhem, the Netherlands
"Single Molecule Mechanics of Nucleic Acids"
D. Chatenay, University Louis Pasteur, Strasbourg, France

Deoxyribonucleotides and Ribonucleotides are the constituents of 2 very important biopolymers (DNA and RNA). Recent experiments performed at the single molecule level allow to investigate their mechanical properties, thus allowing to evidence their structural polymorphism. In the case of DNA the double helix structure can be severely distorted thus leading to new conformations of this double stranded nucleic acid (such as S-DNA and P-DNA). In order to describe such structural transitions one needs to take into account the unique topological nature of this molecule. Furthermore such micromanipulation experiments at the single molecule level give access to the energy costs to go from one structure to another and evidence the fact that DNA is a highly flexible molecule. More recent experiments performed to investigate the interaction between double stranded DNA (dsDNA) and RecA (a bacterial protein involved in DNA repair system and in bacterial recpombination) show that this flexibility might play a role in the binding of proteinsto dsDNA.

In the case of RNA the same kind of experiments can give access to some information about the secondary structure of this single stranded nucleic acid. Such secondary structures are formed by internal hybridation of complementary sequences. Force-extension curves evidence the opening of such internal structures and give information about the energetic cost of their opening. I will describe experiments which are in progress allowing to adress this problem of secondary structure of RNA (RNA folding) both after and during transcription.
"Charged Interfaces: Counterion Condensation and Forces"

P. Pincus, University of California at Santa Barbara; Santa Barbara, USA

We discuss the forces between surfaces with a fixed charges in the context of DNA condensation by multivalent counterions. In particular, we will emphasize the role of non-universal chemical effects on the short-range attractive interactions. The interplay between charge quantization and correlation effects will be central ingredients.
Assemblies of Neurons

"Assembling Neuroelectronic Hybrids"
P. Fromherz, Max-Planck Institute for Biochemistry, Munich, Germany

Abstract:
Bioelectronic interfacing between semiconductors and living cells may lead to sensor chips for pharmacological screening, to actuator chips for modulating molecular signals and cellular growth, and in particular to neuroelectronic devices for neurocomputation and neuroprosthetics. For sake of stability, the interfacing should avoid uncontrolled electrochemical processes at the solid/electrolyte interface. A capacitive interaction between the microelectronics of semiconductors and the microionics of cells requires a tight contact of the solid surface and the cell membrane and appropriate signals in solid and cell that are suitable for mutual stimulation. Three aspects of the problem are considered:

(i) Using molecular electronic probes, we study the gap between a cell membrane and oxidized silicon. From the optical microcavity effect of a fluorescent cyanine dye we obtain a width in the range of 100 nanometer. The electrochromic effect of a fluorescent hemicyanine dye indicates a sheet resistance in the range of 10 megaohm-square. For mammalian cells the time constant of highpass coupling is around 10 microseconds.

(ii) Using genetic recombinant techniques, we insert voltage-gated ion channels into cells on a chip. Fully active potassium channels are observed in the contact region with significant accumulation on the gate of a transistor. This approach will lead to an enhanced ionic current in the junction of small mammalian neurons for controlling electronics and to an enhanced sensitivity of mammalian neurons to electronic stimulation.

(iii) Using large identified neurons from the pond snail, we build elementary neuroelectronic devices. A neuronal loop was assembled with capacitive stimulation from a chip to a neuron, with synaptic transmission in a grown neuronal network and with transistor recording of a second neuron. The formation of networks is controlled by chemical, mechanical and electrical guidance. A silicon microprosthesis is created with transistor recording of neuronal excitation, with signal recognition and amplification on the chip and capacitive stimulation of a second disconnected neuron.
"Progress toward the Design of In Vitro Neural Networks"
B. C. Wheeler, University of Illinois, Urbana-Champaign, USA

Through the use of microstamped patterns of polylysine against covalently linked backgrounds of polyethylene glycol, we have been able to maintain patterns of neurons for up to a month in culture. We have demonstrated the ability to use patterning technology in combination with planar microelectrode arrays to confine the neurons to narrow (10 um or 40 um) tracks which intersect the electrodes and to record spontaneous electrical activity (action potentials) from them. Work is in progress to determine how sparse a network can be and still maintain functional electrical activity. This work is intended to provide a technological basis for robust, repeatable and designable neural networks from which one could study basic neuroscience or construct a neural biosensor. This work is supported by NIH grants R21 NS 38617-01 and R55 RR13320-01and fellowship F30 MH12897. Work presented is from collaboration of the laboratories of the author and Dr. Gregory J. Brewer of Southern Illinois University Medical School.
Nano-Manipulation Techniques

"Forces in Biological Adhesion"
D. E. Leckband, University of Illinois at Urbana Champaign; Urbana, USA

Abstract:
Intercellular adhesive junctions often exhibit defined architectures with distinct protein organization, which is linked to 1) the structures and kinetic properties of the adhesion proteins and 2) the composition and mechanical properties of the lipid membranes. For example, the tight adherens junctions between cells in tissues comprise dense plaques of the adhesion protein cadherin. The equilibrium membrane separation distances are relatively small, being on the order of 200Å. At immunological synapses between immunological cells and target cells, different proteins initially engage the cells, but then sort into organized domains in which proteins segregate at different distances from the central contact and at different intermembrane distances (1).

Such organization results from the interplay of intersurface forces and the dynamics of intermolecular association. We are using the surface force apparatus to quantify the relationship between the structure of adhesion proteins, and the range and magnitude of their interactions within the complex environment of the cell surface. Such direct force measurements can provide fundamental insights into the relationships between protein and membrane composition and the forces that influence the organization of cell-cell adhesive junctions (2).

Our recent studies focused on the adhesion protein cadherin (3). Cadherin is a large, transmembrane protein that binds to identical proteins on adjacent cells. Its extracellular adhesive region folds into five, 45Å long domains. Although it was initially proposed that the proteins bind via their outermost domains (ends), direct force-distance measurements showed that the proteins bind in either of three, distinct antiparallel alignments. The strongest bond involves the fully aligned proteins, and the weakest bond is between the outer domains (3). This is unusual because the majority of receptor-ligand interactions involve single, unique sites on the molecules.

These multiple adhesive interactions may be designed to impede the abrupt rupture of these essential junctions. In force measurements, rather than rupturing abruptly upon bond failure, the proteins pulled apart sluggishly in three stages marked by different separation velocities (3). Upon rupture of the strongest interactions, cadherins appear to become caught, sequentially in the second and third minima before separating altogether. Alternatively, the outer minimum may allow the proteins to bind initially, and to then slowly form stronger contacts at shorter separations as the proteins accumulate in the adhesive junction. Consistent with this, direct force measurements showed that, if the proteins were brought to a separation distance at which only their outer domains engaged, thermal fluctuations would slowly drive the proteins in to the deeper adhesive minimum at smaller membrane separations. The ability to form contacts at different membrane separations may also provide a mechanism for maintaining cell-cell adhesion, despite variations in the steric barriers on cell surfaces. This would allow for some plasticity in the range of intercellular distances at which receptors and ligands engage. This possibility, while untested, is currently being investigated by force-distance measurements of the impact of carbohydrates on inter-protein interactions.

In summary, we have used direct force measurements to determine the relationship between adhesion protein structure and the interactions that contribute to the formation of tight adhesive junctions in tissues. We thus uncovered an unusual binding mechanism, which may play an essential role in both the formation and stabilization of adherens junctions in vivo.

“Optical Tweezers and Three Dimensional Scanning Probe Microscopy”
E.-L. Florin, EMBL; Heidelberg, Germany

Abstract:
That light has a momentum that can be transferred to matter played a significant role only in astronomy until it was realized that radiation forces could be sufficiently large to dominate the gravitational force acting atoms and microscopic particles [1]. The high light intensities required to build traps based on optical forces became available through lasers. The key experiment for the biological application of optical forces was the demonstration that a strongly focused laser beam was sufficient to generate a three-dimensional trap for dielectric particles as small as 25 nm in water and at room temperature [2]. Nowadays, so called “single beam gradient trap” or “optical tweezers” are widely used in biology to manipulate biological material such live cells, and to measure small forces generated for instance by molecular motors [3].

One key feature of optical tweezers is that the range of available forces matches the range of thermal forces, which makes them ideal tools to investigate the mechanical properties of soft material such as lipid membranes [4] or (bio-) polymer networks, to study colloidal forces, and conformational dynamics of single molecules. Conversely, the thermal forces acting on the trapped particle lead to considerable large position fluctuations, making nanometer precise three-dimensional positioning and direct force measurements difficult. Therefore, a detailed analysis and understanding of coupled thermal fluctuations of the force transducer and the sample is essential to the retrieval of quantitative information, and thus most of my talk will be connected to this issue. Specifically, the talk will cover the following topics: (1) Brief introduction to the theoretical description and calculation of forces in a single beam trap; (2) 3-D particle tracking with subnanometer spatial and microsecond temporal resolution; (3) Application of thermal fluctuation analysis to study the three-dimensional molecular mechanics of single motor proteins; (4) Application of thermal fluctuation analysis to study single molecules in complex environments such as a protein in the plasma membrane of a living cell; (5) Application of thermal motion in two- and three-dimensional scanning probe microscopy based on optical tweezers. Finally, I will give a future perspective on applications of optical tweezers and optical tweezers based scanning probe microscopes in molecular and cell biology, soft matter physics, polymer physics, and the study of colloidal forces.

References:
“Using Force to Probe Chemistry of Adhesion Bonds at Cell Interfaces”
E. Evans, University of British Columbia; Vancouver, Canada

Abstract:
Well known in biology, ligand-receptor interactions are the fundament of nanoscale chemistry in recognition, adhesion, signaling, activation, regulation, and a host of other processes from outside to inside cells. Although labeled as bonds, these molecular attachments are each composed of many atomic scale – noncovalent interactions. Thus, the energy landscape of a single adhesion bond can be very complex with several prominent energy barriers that impede kinetics. Usually focused on near equilibrium kinetics, most assays in biological chemistry are only affected by a single-paramount energy barrier. But when bonds are subjected to external force, hidden-inner barriers emerge to set different time scales for kinetics. Probed with ramps of force over an enormous range of rates (force/time), the statistics of bond rupture as a function of loading rate provide a spectroscopic image of energy barriers traversed along the force-driven pathway. In this way, dynamic force spectroscopy is being used to explore energy landscapes that govern lifetime and strength of cell adhesion bonds. Critically important in cell adhesion, bond strength is found to vary enormously and nontrivially with timeframe for detachment. The intriguing question is what are the chemical design principles used by nature to achieve bond strengths appropriate for bioadhesive functions? Not only in adhesive function, design of textured energy landscapes with multiple barriers may optimize dynamic performance of molecular machines and soft material properties or enable structural forces to dynamically signal, switch, and catalyze chemical reactions.


"Transient Pores on Giant Vesicles: Transport, Exocytosis and Fusion"
F. Brochard-Wyart, University Pierre and Marie Curie; Paris, France

Abstract:
We have visualized 1-10 micron sized transient pores in mechanically stretched giant unilamellar vesicles (GUVs). GUVs are artificial liposomes (with diameters of 10-100 microns) used to mimic the lipid envelope of living cells. The membrane of a GUV is a thin fluid bilayer, which has, under ordinary circumstances, almost zero surface tension. By using various different means, we stretch the vesicles, creating tension which is nevertheless far below the usual interfacial tensions of liquids. However, this tension is sufficient to cause major reorganizations of the lipids: the membrane transiently ruptures, allowing the leakage of the inner liquid through a giant pore. The opening of the pore is driven by the surface tension, and its closure by the line tension. We use fluorescent membrane probes and video-microscopy to study the dynamics of the pores. These can be visualized only if the vesicles are prepared in a viscous solution to slow down the leak out of the internal liquid. We have studied the transport of solute, DNA, and small vesicles through these pores. We can reduce the line tension dramatically by adding surfactants to the system. This increases the lifetime of the pores from a few seconds to a few minutes, and the critical surface tension to open a pore becomes very small. Under these conditions we observe spontaneous exocytosis and fusion.
"The Optical Stretcher"
J. Guck, University of Texas at Austin; Austin, USA

Abstract:
All eukaryotic cells depend in their internal structure and organization on the cytoskeleton, a polymer network within the cell interior. These cells reversibly assemble protein filaments (actin filaments, intermediate filaments, microtubules) and accessory proteins into extensive three-dimensional networks. Permanent disruption of this protein network results in apoptosis. More subtle changes can obstruct important cell functions. On the other hand, changes in cell function, such as malignant transformation, feedback into cytoskeletal structure and elastic strength of cells. Changes in the cytoskeleton are key, and even diagnostic, in the pathology of some diseases, including cancer. Existing techniques for measuring the elasticity of cells have a drastic limitation: the tedious sample preparation limits the number of cells investigated per sample, ruling out applications in clinical diagnostics. To accurately measure cell elasticity we have developed an optical tool to stretch single cells between two counterpropagating laser beams exiting single-mode optical fibers. By incorporating the fibers into a microfluidic flow chamber that directs a low-density cell suspension into the trapping region, samples with many cells can easily be measured and sorted. While the total net force on a cell in this two-beam trap is zero, the forces on the surface of the cell can reach up to 400 pN. These deformation forces act on the surface between object and surrounding medium and are significantly higher than the trapping forces on the cells. Radiation damage is avoided since this trapping scenario does not require focusing for stable trapping. Ray-optics was used to calculate the stress profile on the surface of the trapped cell. Measuring the net forces and deformations of well-defined elastic objects validated this approach. We have successfully used the optical stretcher to investigate a variety of cell types, including human erythrocytes, neutrophils, and mouse fibroblasts. Model cell lines were used to explore to what extent cell elasticity is a good parameter to detect cancer cells. We compared NIH3T3-fibroblasts to clonal populations of these cells malignantly transformed by SV40, or h-ras. The transformed cells were either easier to stretch or responded more viscously to the stress. Hence, the optical stretcher seems to be ideally suited for the screening of cell populations in order to detect cancerous cells.

References:
J. Guck et al., Phys. Rev. Lett. 84, 2000
The Extracellular Matrix and the Cytoskeleton – From Model Systems to Reality: The Extracellular Matrix

“Polylipids Anchored to Membranes: Theoretical Aspects”
B. Fourcade, CEA-Grenoble; Grenoble, France

Abstract:
The lipid bilayer of cell membranes is usually connected to two types of biopolymers: The glycocalix and the cytoskeleton. These structures alter the surface properties of the cell. Among the various attempt to mimic the cell properties, one method is to connect polymers to the membrane by anchoring hydrophobic headgroups into the bilayer. In this talk, I will review some of the experimental and theoretical works concerning two aspects. The first deals with the lateral organization of the anchor molecule when they are compatible/incompatible with the phospholipids of the membrane. This includes a description of the polymeric component connected to the membrane. Second, I will discuss some of the morphological changes induced by anchoring the chains into the membrane.
"Pearling Tubulation and Coiling of Phospholipid Membranes by Amphiphilic Polymers"
J. Stavans, Weizmann Institute of Science; Rehovot, Israel

Abstract:
I will summarize the results of an experimental and theoretical study of the morphological instabilities induced by amphiphilic polymers on self-assembled phospholipid membranes of different geometries.

The elasto-mechanical properties of simple phospholipid membranes have been the focus of intense study within the last two decades, as a minimal model of biological membranes. Our study attempts to go a step further, mimicking the effects of macromolecules such as proteins, which are associated with membranes. Our polymers consist of a hydrophilic polysaccharide backbone unto which a number of small hydrophobic groups have been grafted. In solution, these groups anchor on one leaflet of a bilayer, and as a result striking morphological instabilities are observed. Polymers induce (i) pearling in hollow tubular vesicles, (ii) tubulation in highly oblate vesicles, and coiling in cylindrical multilamellar stacks of bilayers. Some of the phenomena we observe have a counterpart in the biological realm.

Our studies have provided evidence supporting the induction of spontaneous curvature by the polymers, as the main mechanism driving the instabilities we observe. Since the bilayers in our experiments are in a fluid-like state, polymers can diffuse along them, to regions of high curvature, and therefore their local concentration and local bilayer curvature are coupled. I will survey the experimental evidence and the statistical mechanical model proposed to account for these observations.

"Oligomerization in the Extracellular Matrix"
J. Engel, University of Basel; Basel, Switzerland

Abstract:
Essentially all cells of an organism are surrounded by extracellular matrix (ECM), which mediates cell communication, cellular differentiation, cell migration and maintenance of tissues. The ECM is a network of manifold interlinked multifunctional proteins and polysaccharide chains. ECM proteins are composed of many domains and are usually very large. Network formation occurs by interactions of multistranded oligomerization domains as collagen triple helices and a-helical coiled-coil domains. The thus formed multimers interlink with specific domains of the multidomain proteins. Cellular receptors on plasma membranes are also involved in network formation. A mechanical connection exists to the cytoskeleton via membrane spanning receptors.

Oligomerization leads to functional advantages of multivalency and high binding strength, increased structure stabilization and a combined function of different domains. These features seen in naturally occurring proteins can be engineered by protein design by combining oligomerization domains with functional domains. Examples are the assembly of laminin to basement membranes (1), the interaction of polyvalent ligands with receptors which are activated in a trimeric form (2), the stabilization of triple helices by cross-linking with oligomerization domains (3), and the homophilic interaction of adhesion proteins on a membrane surface (4).

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Frank S., Kammerer R. A., Mechling D., Schultess Th., Landwehr R., Bann J., Guo Y., Lustig A., Bächinger H. P.,


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A.A. Antipov, G. B. Sukhorukov, S. Leporatti, E. Donath and H. Möhwald  
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2) Laboratoire d' Enzymologie et Biochimie Structurales, CNRS  

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Max-Planck Institute of Biochemistry  

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