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BLOCKBUSTER HOLIDAY ISSUE!!

The Electrosensory Landscape: How Fish "See" Electric Fields
by Dr. Brandon Brown

We have recently turned our attention to electrical signal development in the electrosensitive organs of sharks and their relatives. We ask how the material and geometrical/morphological properties of the sense organs affect their function, and this question has taken two forms: one pursuing the measurement and electrical characterization of substances within the organs; and another seeking to explore the effect of whole-body morphology on the electric sense via computational modeling. Here, we sketch both investigations.

Properties of glycoprotein gel

Our experimental investigation lives at the intersection of soft condensed matter, polymer physics, sensory biology, and biochemistry. We seek to understand the role of a sophisticated biological gel in the electrosensory organs of sharks.

Elasmobranchs (sharks, skates, and rays) are known to passively sense tiny (< 5 nV/cm in some species) variations of ambient electric fields using organs known as the ampullae of Lorenzini.[1] Marine elasmobranchs possess hundreds of these ampullae, and the electric sense has been shown to facilitate prey location, navigation, and mate identification. The ampullae of Lorenzini are also found in the paddlefish, *Polyodon spathula*, an electrosensitive predator. Recent groundbreaking work in biophysics has explored the role of noise in that system.[2]

The ampullary organs are innervated bulbs beneath the skin; the ampullae are connected to the aqueous environment by long canals, and a clear extracellular gel fills each canal-ampulla system (see Figure 1). To date, the canals and their relatively conductive gel ($\rho \sim 25$ Ωcm) have been treated as voltage contacts between an elasmobranch's environment and the sensing ampullae. We seek to map the transport properties of the gel to better understand the functioning of the electrical sense organs. Could evolution have fine-tuned the gel's transport properties?

Historically, the extracellular gel has received very little attention; what is known can be quickly summarized. The gel contains 97% water by mass, with the remaining 3% made up of large, heavily sulfated glycoprotein molecules. In addition, the gel is rich in dissolved salts, sensibly approximating the concentrations found in seawater.[3] The exact structure of any large glycoprotein is difficult to ascertain, and whether the large gel polymers are best classified mucopolysaccharides or glycos-aminoglycans is still an open question.
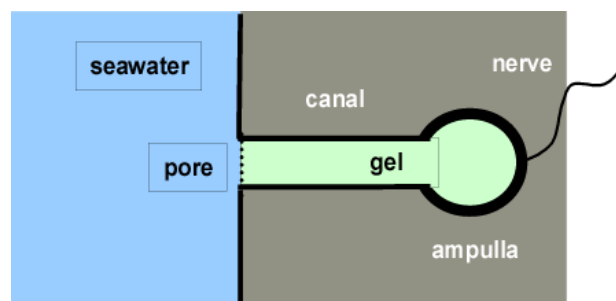

Figure 1: Highly simplified cross-section of an electroreceptive organ: the ampulla of Lorenzini and its associated canal. Sensing cells line the ampullary wall. The width of the canal (typically 1-2 mm across) is exaggerated here with respect to its length (typically 5-20 cm).

Over the last 18 months, we have collected gel from newly deceased shark specimens. Two of these, *Triaenodon obesus* (white-tip reef shark) and *Carcharinus melanopterus* (black-tip reef shark), were obtained from the Steinhart Aquarium at the California Academy of Sciences, and another (a 15-ft *Carcharodon carcharias*, white shark) was obtained with the cooperation of the Pelagic Shark Research Foundation.

To date, we have applied a diverse battery of measurement techniques to the gel, including four-terminal DC electrical transport, impedance spectroscopy, and polyacrylamide gel electrophoresis.[4] We apply these techniques to both unaltered samples and dialyzed samples that have been leached of dissolved salts. In addition, we have started collecting noise spectra and thermopower measurements.

We first tested the gel for electric and/or magnetic field dependence. In our University of San Francisco laboratory, we use a four-terminal, pulsed DC technique to measure resistivity from 1 nV/cm from 1

V/cm and from 0 gauss to 30 gauss. As reported at the March 2001 APS meeting in Seattle (http://www.aps.org/meet/MAR01/baps/abs/S773000 6.html) none of the species have shown evidence of such dependence. Figure 2 displays the Ohmic behavior for the gel of two species. Noise dominates the data below 100 nV for the DC measurements, as the platinum-to-gel interface effectively forms a Schottky barrier. This leaves open the possibility that the resistivity changes in the range of the organism's sensitivity threshold (below 5 nV/cm).
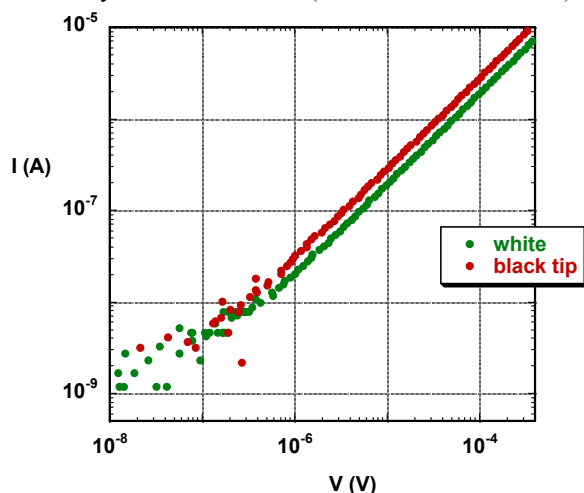

Figure 2: Low-field I-V curves collected from the ampullary gel of two shark species. The Ohmic data extend to higher fields as well (at least 1 volt/cm).

Though results are negative for field-dependent resistivity, the effects of temperature variation are pronounced. In keeping with other electrolyte systems, the resistivity drops appreciably with small temperature increases. We fit all data in an Arhennius format to thermally activated transport, obtaining activation energies of approximately 16.5 kJ/mol for all samples. This corresponds to a proton-transfer transport mechanism, again common to electrolyte solutions and gels alike.

As we have collected more gel from the large white shark than the other species, we were able to subject a 3 ml portion of the white shark gel to dialysis. The sample was held in a micropore membrane pouch and immersed in two sequential one-liter deionized water baths for a total of 48 hours. By volume comparison, we estimate the concentration of dissolved salts to have dropped to roughly 1×10^{-5} of the original level. The resulting resistivity of the dialyzed gel increased by a factor of 50 over the native gel. Interestingly,

the volume fell to one-third its native value, even though the large glycoproteins remain, and the structural elasticity of the gel vanished. What is naturally a cohesive, robust gel becomes formless

and plastic when the dissolved salts are removed. Such structural changes in polymer gels have been explored in detail elsewhere.[5]

To examine the frequency-dependent properties of the gel, we have recently collected preliminary impedance spectra from both the native and ion-depleted (dialyzed) white shark gel samples. John C. Hutchison, a physical chemist in Royce W. Murray's laboratory at the University of North Carolina – Chapel Hill, has collected a series of impedance spectra showing very large real components of the dielectric constant for the low frequencies corresponding to the operational range of the sense organs (~ 0.1 Hz – 10 Hz).[4] We hope to continue these measurements until we have a detailed map of the gel's complex permittivity.

In the face of these electrical measurements, a practical question remains: how might the gel be altered post mortem or upon removal from the electrosensitive organs, and how does it age in the laboratory? While we have not been able to measure gel properties in situ, we now have strong evidence that the basic composition and structure of the gel is robust in the face of the thermal and electrical cycling encountered during experiments. This evidence appears in the results of polyacrylamide gel electrophoresis (see Figure 3). Douglas R. Kellogg, a biologist at the University of California, Santa Cruz, has carried out the electrophoresis trials. Electrophoresis stains the central proteins in the glycoprotein molecules (here, we use Coomassie staining) and places a uniform charge on them before letting them "run" between an anode and cathode. In this way, the components separate by mass. In Figure 3, the results of three species tested immediately after collection are shown side-by-side with the results of white shark gel tested after copious transport experimentation (including trips to and from a storage freezer at –20 C) and the dialyzed sample. The multi-component, hazy bands are typical of large glycoproteins. Though little similarity is found between the species, it is striking to note the consistency for the white shark gel, before and after experiments, freezing, and even after dialysis. We interpret these results to mean that the constitution of the glycoproteins is robust in the laboratory over time. However, the exact structure and composition of the component molecules have yet to be determined, and our samples may differ from the gel in a live sense organ.
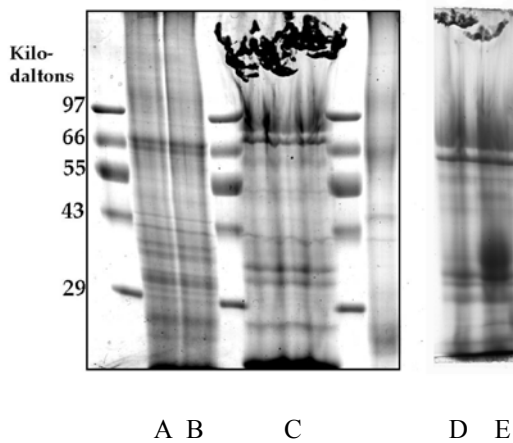
Figure 3: Electrophoresis results for five samples of shark gel collected from the electrosensitive ampullae and associated canals. The sharp numbered bands denote protein standards. (A) white-tip reef shark, (B) white shark, (C) black-tip reef shark, (D) post-dialysis white shark, and (E) white shark after many experimental trials.

The origin of the high-mass (see the top of Figure 3) anomaly in the white-shark trials is unknown at this time, but it is as reproducible as it is perplexing. Since the glycoproteins are known to be highly sulfated in this gel, the anomaly might denote very long, massive molecules that also contain a substantial inherent charge.

In the months ahead, we hope to augment the measurements described above. Since noise has been shown to be crucial to the firing of the neurons associated with the electrosensitive organs,[6] we have started collecting detailed noise spectra from the gel in a variety of electrical and thermal environments. In addition, as the electrosensors of elasmobranchs have also shown incredible sensitivity to temperature, we consider an investigation of the thermopower in the gel to be an exciting and logical next step.

Modeling the electrosensory landscape
In addition to our laboratory experiments, we computationally model the electrosensory input of an elasmobranch moving near prey.[7] Though many studies have mapped the neuron firing rates resulting from various electrical stimuli on a single ampulla,[8] little or nothing is known about how an elasmobranch uses the multiplicity of its electrosensors to reconstruct the relevant perturbations (e.g. prey) to its environment. Our modeling efforts hope to address this.

We start with the case of a prey fish modeled as a DC electric dipole. (This assumption has proven to be an excellent approximation for the bioelectric field of a small fish in various behavioral experiments, [1]). Using basic electrodynamics, we can calculate the low-frequency voltage signals that develop along the array of ampullary canals as a predator moves with respect to its dipole prey. The canals lie in various positions and cover a wide range of orientations within an elasmobranch, so the resulting signals vary accordingly.

By giving us a window into the electrosensory landscape of these creatures, our calculations bear directly on both observed feeding behaviors and also comparative morphologies. The latter is especially intriguing as the elasmobranchs have evolved a notable array of divergent shapes.

In our first effort, we use a simple and symmetrical pair of two-dimensional elasmobranch models (a rounded rostrum versus a hammer-shaped rostrum).[7] The analysis is also, to date, fairly rudimentary. Efforts are now underway to flesh out these skeletal efforts, utilizing the expertise of Marcelo Camperi, a computational physicist at the University of San Francisco. We will now employ models that reflect the exact canal and ampulla geometry of dissected elasmobranchs,[9] and we hope to move beyond the basic voltage signals in the sensing organs to a more complete neurodynamical analysis. Recent exciting work on arachnid prey location has arrived at a complete picture using a neuron population vector analysis.[10] Where a sand scorpion uses vibrational data from eight inputs (legs), the elasmobranch utilizes electrical data from hundreds of ampullae.

An informal website for the laboratory exists at: www.usfca.edu/physics/brandon.htm.

References (these are incomplete, but certainly give landmarks and launching points into the literature).

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SCIENCE AND SOCIETY
Fighting for Evolution:
The National Center for Science Education
by Glenn Branch
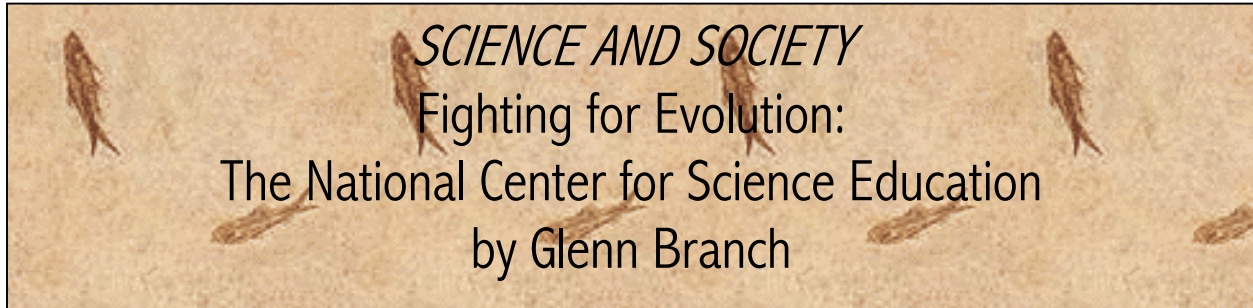
Biological physicists like to live in an ivory tower as much as any other scientists. Yet, today, as throughout history, science and politics are inextricably intertwined. In the first of an occasional series of "SCIENCE AND SOCIETY" columns, The Biological Physicist has invited the following commentary from Glenn Branch, of the National Center for Science Education. NCSE is a watchdog group that monitors creationist attempts to influence science education in the United States.

■ *SB*

Were you among the millions of people who watched the recent PBS series *Evolution* (broadcast on September 24–27, 2001)? If so, you probably watched episode 7 — "What About God?" — with amazement, as Ken Ham, the executive director of the antievolutionary ministry Answers in Genesis, encouraged children to respond to their science teachers' assertions about events in the prehistoric past with an insolent "were you there?" Isn't religiously motivated opposition to the theory of evolution a thing of the past? Wasn't it dispelled for once and for all with the Scopes trial in 1925?

No such luck. Creationism is alive and well, and now available in a dizzying array of varieties, depending on the particular theological predilections of its adherents. What unites creationists, whether they style themselves young-earth creationists, old-earth creationists, or intelligent-design creationists, is ideological opposition to the theory of evolution, regardless of the overwhelming evidence in its favor and regardless of the virtually universal acceptance of it by the scientific community. Unfortunately, creationists are not content to keep their doubts to themselves. In fact, just in 2001, measures intended to weaken evolution education — either legislation or state educational standards — were introduced in no fewer than ten states, containing about twenty percent of the nation's population. Creationism is a genuine threat.

Founded in 1983, the National Center for Science Education (NCSE) is the only organization wholly devoted to countering the creationist threat to evolution education. We do so on several fronts. We help to educate the public at large though our publications, including our bimonthly journal *Reports of the NCSE* and our books *Voices for Evolution* and *Reviews of Creationist Books*, through our website, www.ncseweb.org, and through the media, which increasingly recognizes NCSE as the definitive source of reliable information on evolution education and creationist attacks on it. And at the grassroots level, we provide information and advice to teachers, parents, and concerned citizens facing local creationist challenges in their communities.

If creationism comes to your community, there are three lines of argument that you are sure to encounter. These are, as it were, the three pillars of antievolutionism.

Pillar 1. "Evolution is a theory in crisis." This assertion is typically supported by the following sorts of claims. (A) Assertions that evolution is incompatible with established results from other

branches of science, such as the perennially popular contention that evolution is precluded by the second law of thermodynamics. (A bizarre theological addition is that the second law is due to original sin!) (B) Misquotations or quotations out of context from scientists that appear to disavow evolution. The biophysicist Harold Morowitz, for example, once calculated the odds of life's originating in a state of thermal equilibrium as $10^{-339,999,866}$; it was not long before creationists started to cite his calculations as evidence for the improbability of a naturalistic origin of life, despite the fact that the earth has never been in a state of thermal equilibrium. Such quotations, by the way, are often hugely out-of-date; Morowitz's calculation dates from 1968, and is still misused. (C) Misrepresentations of scientific controversies about the mechanisms or the patterns of evolution as controversies about the occurrence of evolution. For example, intelligent-design creationists have recently claimed that Stuart Kauffman's work on self-organizing systems is a challenge to evolution, causing Kauffman to respond "there is nothing in my work that I personally take to support creation science, if by some stretch of the definition it be science at all. "

Pillar 2. "Evolution is antireligious." It is of course true that evolution is at odds with the religious beliefs of creationists. Eager to find allies, however, creationists frequently further assert that evolution is incompatible with religion — or Christianity — *tout court*. (Moreover, there are zealous atheists who agree, although they draw the opposite conclusion, arguing that atheism follows from evolution.) But there are plenty of scientists who are both devout Christians and ardent evolutionists; a recent example is Brown University's Kenneth R. Miller, whose *Finding Darwin's God* (HarperCollins 1999) fiercely castigates creationists and eloquently describes the support for his faith that he finds in evolution. People like Miller are not necessarily flouting the positions of their churches, either: of Americans in the largest twelve Christian denominations, over 75% belong to churches that support evolution education. NCSE's publication *Voices for Evolution* reproduces statements endorsing evolution education from a dozen religious denominations. In fact, evolution education is no more antireligious than, say, blood transfusion: both, regrettably, incur religiously motivated opposition, but there is good secular reason for both.

Pillar 3. "It is only fair to teach both sides." A recent variation is embodied in the slogan "Teach the controversy," the point of which is to suggest that teaching both evolution and creationism improves critical thinking skills. As important as such skills are, they are not enhanced by their exercise in what is

a scientifically uncontroversial arena. Just as the claims of Holocaust deniers should not be taught to "balance" a class in modern history, the claims of evolution deniers should not be taught to "balance" a class in biology. For (as readers of *The Biological Physicist* are well aware) science is not a democratic process. What is fair — both to the students and to the teachers — is to ensure that what is presented in science classes is what is accepted by the scientific community, not what a particular religious pressure group prefers. To do otherwise is to cheat the students of their chance to become scientifically literate members of our society.

So if creationism comes to your community — and don't assume that it never will, or that it hasn't already — be prepared to explain what is wrong with the three pillars of antievolutionism. And call us at NCSE. We can help.

To get more information about NCSE, or to join, write to NCSE, Box 9477, Berkeley CA 94709-0477, call 1-800-290-6006, send e-mail to ncse@ncseweb.org, or visit the NCSE website at www.ncseweb.org. NCSE is a 501(c)(3) nonprofit organization.

Some Recommended Reading

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From cDNA Microarrays to Genetic Networks: Theory and Practice at KGI
by Dr. Greg Dewey

Much has been written about the genomic revolution and how it is transforming biology. With the aid of high-throughput technologies, the sequencing of various genomes has generated data at an exponential pace for over 6 years. These efforts have created enormous databases and have brought computational biology to the forefront. The combination of microscale biochemistry, laboratory automation and computational power has not only changed how biology is done but has also altered how fundamental biological problems are approached. In this era of "systems biology", we seek to quantitate the level of expression of all the genes in the genomes (functional genomics) and the production of the entire repertoire of proteins (proteomics) in response to various cellular perturbations.

The cDNA microarray technology has received considerable recent attention for its promise in quantitating the mRNA level of all the genes in a genome. This "lab on a chip" method measures mRNA levels using selective hybridization of RNA (or cDNA) to a cloned DNA sample spotted on a glass slide. This measurement reveals which genes are active and producing messengers and which genes are turned off. Approximately, 3000 measurements can be made from a square centimeter of a glass slide. This new high throughput technology can be viewed as a "second generation" technology to the genome projects and is a natural extension of them. The genome projects provide us with a list of genetic parts for an organism while the microarray technologies tell us which parts are in use at a given point in time. One of the emerging central problems in this field is to use the whole genome expression profiles to generate a gene regulatory network. This would provide a causal map of the interactions and control of the system.

At the Keck Graduate Institute (KGI) in Claremont, CA, an interdisciplinary faculty is being assembled to tackle problems in systems biology. KGI was founded in 1997 and is the newest member of the Claremont University Consortium. It is a research-intensive institute that seeks to train professional masters students for the biotech and pharmaceutical

industry. We are also developing an innovative PhD program that combines computational science, bioengineering and systems biology. At KGI, there is a coordinated effort to utilize microarray technologies to attack systems-wide problems in biology. Miguel Barbosa, a cellular biologist at KGI, is using expression profiling to dissect the molecular events in signal transduction in a mammalian cell line that is used as a model system for arthritis. Animesh Ray is a molecular biologist that is interested in the genetic network architecture of yeast. Using molecular biological techniques, he can change the nodes and edges in this architecture and explore the robustness of the biological network. The yeast model system is also used by James Cregg for exploring the genetic regulatory events associated with a morphological change, the formation of peroxisomes, an organelle in certain yeast strains. These experimental efforts are complemented by theoretical and computational work by Greg Dewey, David Galas and David Wild. David Wild has developed hidden Markov models to describe the dynamics of gene expression. Dewey and Galas have explored network models of gene expression derived from simple dynamical models.

The close ties between experimentalist and theoreticians allow the development of experimental designs that ease the interpretation of the data. To date, the bulk of the effort at KGI has been on time-series expression measurements. Typically, one starts with a cellular population in some steady state, perturbs the systems and monitors the response in the expression levels of all the genes. The perturbation can be a wide variety of things and depends on the problem of interest. Typical perturbations are a shift in metabolites or exposure of the cells to a hormone. To date, the biological community has not performed many time-series experiments and this is largely due to the expense of the experiment. Often simple comparisons are performed. Plus/minus drug or plus/minus disease are common experiments. However, time series data naturally lends itself to a mechanistic interpretation and provides a more direct path to mechanistic detail.

A number of dynamical models have been explored to analyze public-domain time series data involving

expression in yeast (1-4). Interestingly, several different investigators have suggested that simple linear models can accurately represent the data. Recently, Dewey, Galas and Ashish Bhan, a mathematics graduate student at Claremont Graduate University, have used autoregression models to analyze expression time series. In its simplest form, this model is given by: $a_i(t) = \sum_j \lambda_{ij} a_j(t-1)$ where

$a_i(t)$ represents the mRNA level of the ith gene at time t and λ_{ij} is a member of the Markov transition matrix. The Markov transition matrix is calculated using singular value decomposition to perform a generalized matrix inversion. The advantage of this formalism is that it leads directly to a network graph that shows the influence of one gene on the production of another. When the absolute value of λ_{ij} is above a certain threshold the entry is given a 1, below the threshold, the entry is given a zero. This procedure produces a sparse adjacency matrix representing a digraph of the interactions between genes.

Figure 1 shows an example of graphs created at different thresholds-the small graph is at a high threshold and the larger graphs are at lower threshold. Each node in the graph represents a gene and an arrow between genes represents the phenomenological influence of one gene expression level on another. In the figure we have only displayed the subgraph that contains the strongly coupled or fully connected nodes. Notice the hub-like structure with central genes surrounded by genes with few connections. This basic structure is seen in all the biological data that we have analyzed. These graphs have certain "small world" features-they have short mean pathlengths (a measure of connectedness) and high cluster coefficients (a measure of cliquishness). In addition to these properties, the graph shows a scale-free distribution of connectivities. That is the number of nodes with k degree, $N(k)$, follows a power law with: $N(k) \sim k^{-\gamma}$. This scaling is independent of the threshold parameter and in all the yeast data that we have analyzed the exponent $\gamma = 3/2$.

In recent years, there has been significant interest within the physics community in large real world networks. Networks, such as power grids and the Internet, often show small world behavior along with a scale-free distribution of connectivities. A number of statistical models based on network growth have been explored to explain the properties of these networks (for a review see 5). These real world networks differ in a very significant way from the

networks that are derived from microarray data. The biological systems never show a scaling exponent greater than 2, while most other large networks show exponents greater than 2. The scaling and small world behavior of the biological systems is difficult to model using existing network growth models. Recently, the KGI group has explored a number of biologically motivated network growth models that do give the proper global network properties (6). These models provide clues to how genetic networks grow and how members in the network are related to each other. The computer simulation and analytical work on these growth models is the subject of ongoing research at the Keck Graduate Institute.
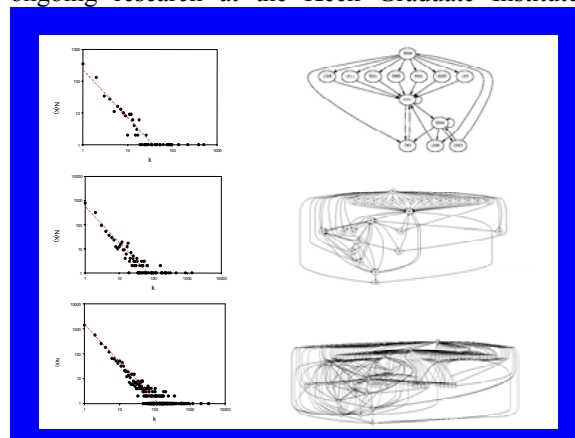

Figure 1. Plots showing the effect of the threshold parameter on the size of a network and on the scaling of node degrees. Strongly coupled components of networks at various thresholds (right hand side). Plot of degree distribution, $N(k)$ versus k for diauxic shift data at different thresholds (left hand side). Plots show that scaling is independent of threshold value. Actual thresholds differ somewhat between left and right hand examples. Threshold was varied by a factor of 2 to generate scaling plots. The lines in right-hand plots represent functions with a slope of -3/2. Note that they intersect the axes at different points, however, reflecting the different numbers of nodes in the graphs.

The genomic era provides a number of challenges to the biophysicist. For the first time, there is systems wide data that begins to reflect the full complexity of biological systems. Yet this data is often not of the statistical quality that one expects from physical systems. The challenge is to develop analysis and modeling techniques for the new systems biology. There is a spectrum of approaches that can be taken. At one extreme are the purely statistical methods such as cluster analysis and principal component analysis. Detailed physico-chemical models that include all the interactions and rate processes of the system are at the other extreme. The statistical methods do not lead to causal models and the detailed

models are not supported by the quality of the data. A third approach is to start with a bare-bones "toy" models that capture the salient features of the system and to then expand it as more information is obtained. This approach requires great intuition into the problem and is not easily generalized. Models that generate graphical networks offer promising alternatives. These models can be statistical in nature yet can have causality built into them in a phenomenological way. Adjacency matrices are computationally easy to manipulate and provide facile means of comparing one network with another. While it is unclear which approaches will be most successful for systems biology, what is clear is the need for investigators with physical intuition and experience in complex systems to work directly with experimental biologists. This is the environment that we are fostering at KGI.

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March Meeting Update!!

In the last issue, we brought you a list of the DBP Focus Sessions for the upcoming March Meeting. In this issue, we have a list of the DBP Minisymposia and Tutorials. For more information, see the DBP website (http://www.aps.org/DBP) or contact Bob Austin, this year's DBP Program Chair (austin@Princeton.EDU).

DBP TUTORIAL

T4 Methods of Nonlinear Dynamics in Cellular Biophysics
Who Should Attend: Those interested in an overview of nonlinear dynamics and pattern formation, with special emphasis on issues related to biophysical phenomena in the realm of molecular and cellular biology.
Course Description: A wide range of problems in molecular and cellular biophysics involve dynamical phenomena of elastic objects, such as membranes, filaments, and flagella. This course will offer a broad yet concise introduction to many of these biological problems of interest to physicists (supercoiling of twisted filaments, self-propulsion, instabilities); the physical considerations (elasticity theory, slender-body hydrodynamics, etc.) underlying their description; computational aspects relating to the associated nonlinear formulations; and emerging methods for experimental studies on model systems.
Organizer and Instructor:
Raymond E. Goldstein, University of Arizona
Instructors:
Chris H. Wiggins, Columbia University
Thomas R. Powers, Brown University
Greg Huber, University of Massachusetts, Boston

SYMPOSIA

Monday AM The Physics of Single Molecules: Nanopore Approaches. Speakers: David Deamer, John Kasianowicz, Amit Meller, Mark Akeson and Daniel Branton

Monday AM Biological Molecules in Solvent Free or Minimal Solvent Environments. Speakers: Jack Beauchamp, Michael Bowers, Mary Rodgers, Evan Williams and Martin Jarrold

Monday PM Statistical Biophysics (Prize Symposium). Speakers: Carlos Bustamante (prize recipient), Naama Barkai, Anirvan Sengupta, Hao Li.

Monday PM Enzymatic and Transcriptional Networks. Speakers: Boris Shraiman, Mark Goulian,

Terry Hwa, Michael Elowitz and Jose Manuel Gomez Vilar.

Tuesday AM Bioimaging: Brain to Genome. Joint symposium with **FIAP**. Speaker list not yet available.

Tuesday PM Rheological Properties of Bio-polymer Networks. Joint symposium with **DPOLY**. Speakers: Fred MacKintosh, Laurent Bourdieu, Christoph Schmidt, Paul Janmey and Jay X. Tang.

Wednesday AM Complex Real World Networks. With **GSNP**. Speaker list not yet available.

Thursday AM Physical Principles of Amyloid Aggregation. With **DCMP**. Speakers: Christian

Ionescu-Zanetti, Ron Kopito, Sue Lindquist, Rahul Kulkarni and Peter T. Lansbury.

Thursday AM Proton Translocation and Buried Charge Formation in Protein Folding and Functioning. Speakers: Denis Rousseau, Pal Ormos and Aihua Xie.

Thursday AM Physics of Protein Folding. Speakers: Cecilia Clementi, Albert-Laszlo Barabasi, Jayanth R. Banavar, Kevin W. Plaxco and Marek Cieplak.

The biomolecular force spectroscopy laboratory at University of Missouri-Columbia by Dr. Michel Grandbois

Thanks to the development of nanomanipulation techniques such as optical and magnetic tweezers, micropipette and scanning probe microscopy, it is now possible to follow and measure the forces involved in complex single molecular events. Our work at the Biomolecular Force Spectroscopy Laboratory **(http://web.missouri.edu/~physwww/ html/faculty.html)** is directed toward the development of experimental and theoretical tools for the manipulation and characterization of single biological molecules and assemblies. The atomic force microscope (AFM) is the platform of choice for our studies due to its high sensitivity in force and position measurements. Forces associated with single biomolecular recognition events and mechanical properties of a variety of biopolymers are now routinely measured using the AFM. Such experimental approaches become particularly relevant for biomolecules or assemblies involved in mechanical role such as cell adhesion, tissue compliance, structural network and biomolecular motors.

Force spectroscopy
Mechanical properties of biopolymers including polysaccharides, proteins and DNA [1] can be

investigated at the single molecule level using AFM based force spectroscopy. In a typical force spectroscopy experiment, a polymer sample is left to adsorb on a flat solid surface and individual polymer segments of random length are picked up by adsorption or by specific attachment- by the AFM tip. The newly formed molecular bridge is then stretched with the help of a precise piezo-electric positioner, capable of nanometer displacement. The cantilever deflection detected upon stretching the molecular bridge generates force-extension curves which reveal a wealth of fingerprint-like features, such as entropic elasticity, conformational transition and supramolecular rearrangements. At low to moderate extensions, most of the biological polymers studied to date can be described by statistical mechanics models of ideal chains (thermally driven random walks), such as the non- extensible and extensible freely jointed chain (FJC) and worm like chain (WLC) [2] models. By fitting experimental data to these equations, it is possible to separate the entropic and enthalpic components of the polymer deformation. Our research in this field is directed toward the understanding of the mechanical properties of biomolecules or biomolecular assemblies responsible for the mechanical integrity of

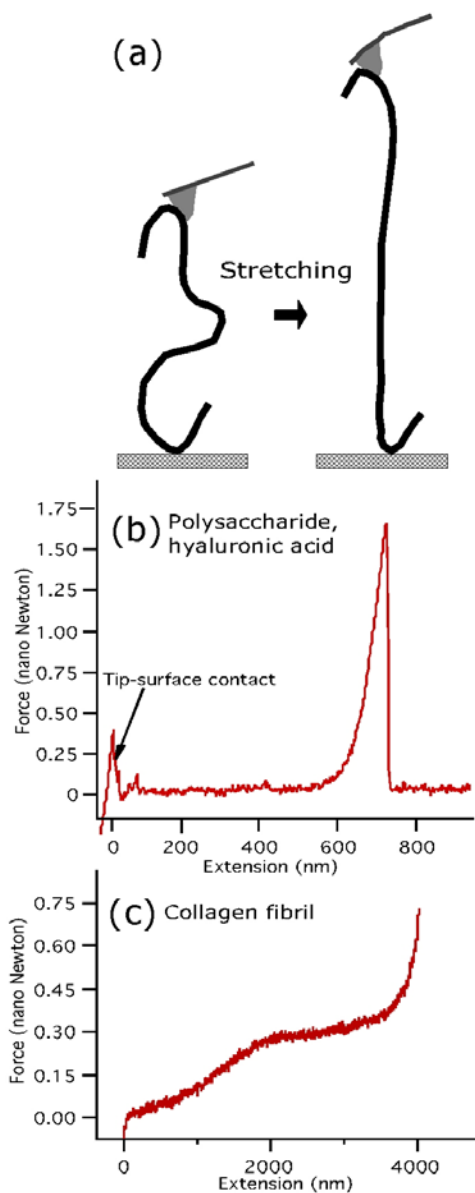connective tissue. Biological molecules or assemblies such as polysaccharides, collagen, elastin and fibrillin are investigated both at the monomer and fibrillar level. Figure 1 presents two typical force-extension urves recorded on a single polysaccharide molecule (hyaluronic acid) and on a single collagen fibril (r=70nm). These two curves reveal a very rich and complex elastic behavior with several distinct elastic regime that can be describe by combining modified entropic model of polymer elasticity with Monte Carlo simulation and molecular dynamics simulation. Our single molecule force spectroscopy experiments pave the way to the full understanding of the biomechanical properties of elastic tissue which is crucial not only for the understanding of various cellular processes but also for the design and engineering of novel materials.

Figure 1. A schematic of a single molecule force spectroscopy experiment is presented in (a). The molecule is attached between an AFM tip mounted at the end of a soft cantilever that acts as a force transducer and stretched with the help of a precise piezo-electric element. Typical force-extension relationships for the polysaccharide hyaluronic acid (b) and for the in-vitro assembled collagen fibril (c) are presented. The curves reveal very rich elastic behaviors and can be analyzed with model for polymer elasticity. The low force regime is generally attributed to a general entropic restoring force. The moderate to high force regime provide information on enthalpic contribution such as conformational changes, unfolding, melting and chemical bond deformation.

Molecular adhesion and affinity imaging
AFM-based force measurement techniques can be used to measure the adhesion force between a biomolecule attached to the AFM tip and another molecule attached to a surface (Figure 2). Polymer spacers between the AFM tip and the receptor are used in order to avoid restrictions of spatial accessibility. The major outcome of our investigations was to clearly demonstrate that a given lectin binds different sugar groups with different forces and that lectin receptors known for their mechanical role in cell-cell adhesion can withstand higher force than receptors involved solely in biosignaling [3]. Extending further the idea of measuring the adhesion between single biomolecular pair, an AFM tip functionalized with a specific biomolecule was used to map the distribution of a specific receptor present on the surface of living cells. We have produced an affinity image of a mixed layer of group A and O red blood cells (RBC) in which the contrast is based only on specific molecular recognition events [4]. The image of our model system was obtained by measuring and plotting for each image pixel the adhesion force (as measured in Figure 2a) between an AFM tip functionalized with helix pomatia lectin and the mixed RBC layer. The high specificity of the lectin for the N-acetylgalactosamine terminated glycolipids present on the membrane of group A red blood cells allowed discrimination between the two cell populations and to produce an affinity map of the cells surface (Figure 2b). We have then, developed a new kind of imaging based solely on specific molecular recognition. Aggregation of membrane receptor and microdomain (also know as rafts) formation within the plasma membrane is believed to play a fundamental role in the regulation of various cellular activities. Considering the perceived ubiquity of membrane rafts as a cellular control mechanism, it is essential to perform experiments that can assess the aggregation state of receptors on non-perturbed living

cells. AFM based affinity imaging is currently applied by our group to the study of membrane raft in order to characterize their physical and biological function.
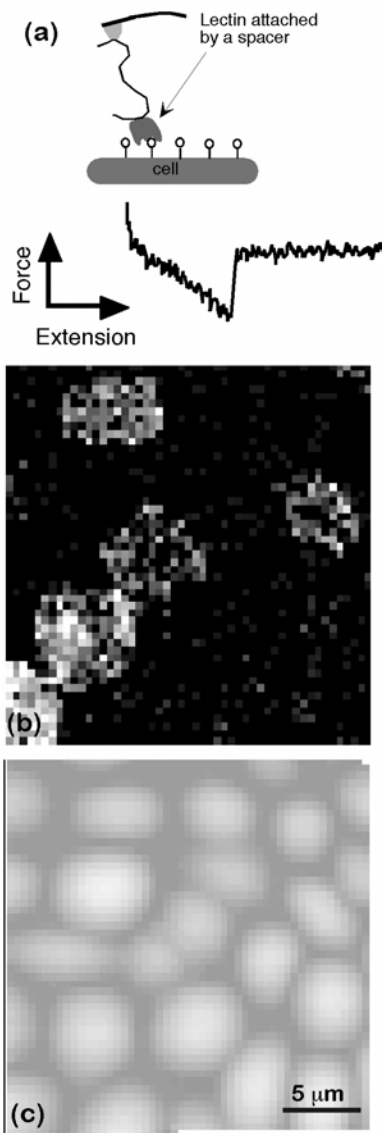

Figure 2. Imaging of layer of mixed (50:50) group A and O red blood cells using an atomic force microscope tip functionalized (a) with a sugar binding protein specific to receptors present at the surface of group A cells. The affinity image (b) is obtained by scanning the cells layer pixel by pixel and by measuring the molecular adhesion force as measured in (a) is plotted on a gray scale to produces the contrast between the two red blood cells. Bright regions correspond to group A cells. The figure (c) a simple cantilever deflection image is presented for comparison.

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References:

1. Rief, M. et al. *Science* **275**, 1295-1297 (1997), Grandbois, M. et al. *Science* **283**, 1727-1730 (1999), Oberhauser et al. *Nature* **393**, 181-185 (1998), Rief, M. et al. *Nature Struc. Biol.* **6**, 346-349 (1999).

2. Smith, S. B. et al. *Science* **258**, 1122-1126 (1992), Bustamante, C. *Science* **265**, 1599-1602 (1994), Ortiz, C. et al. *Macromolecules* **32**, 780-787 (1999).

3. Florin, E-L. et al. *Science* **264**, 415-417 (1994), Dettmann W. et al. *Arch. Biochem. & Biophys.* **383**(2), 157-170, (2000).

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