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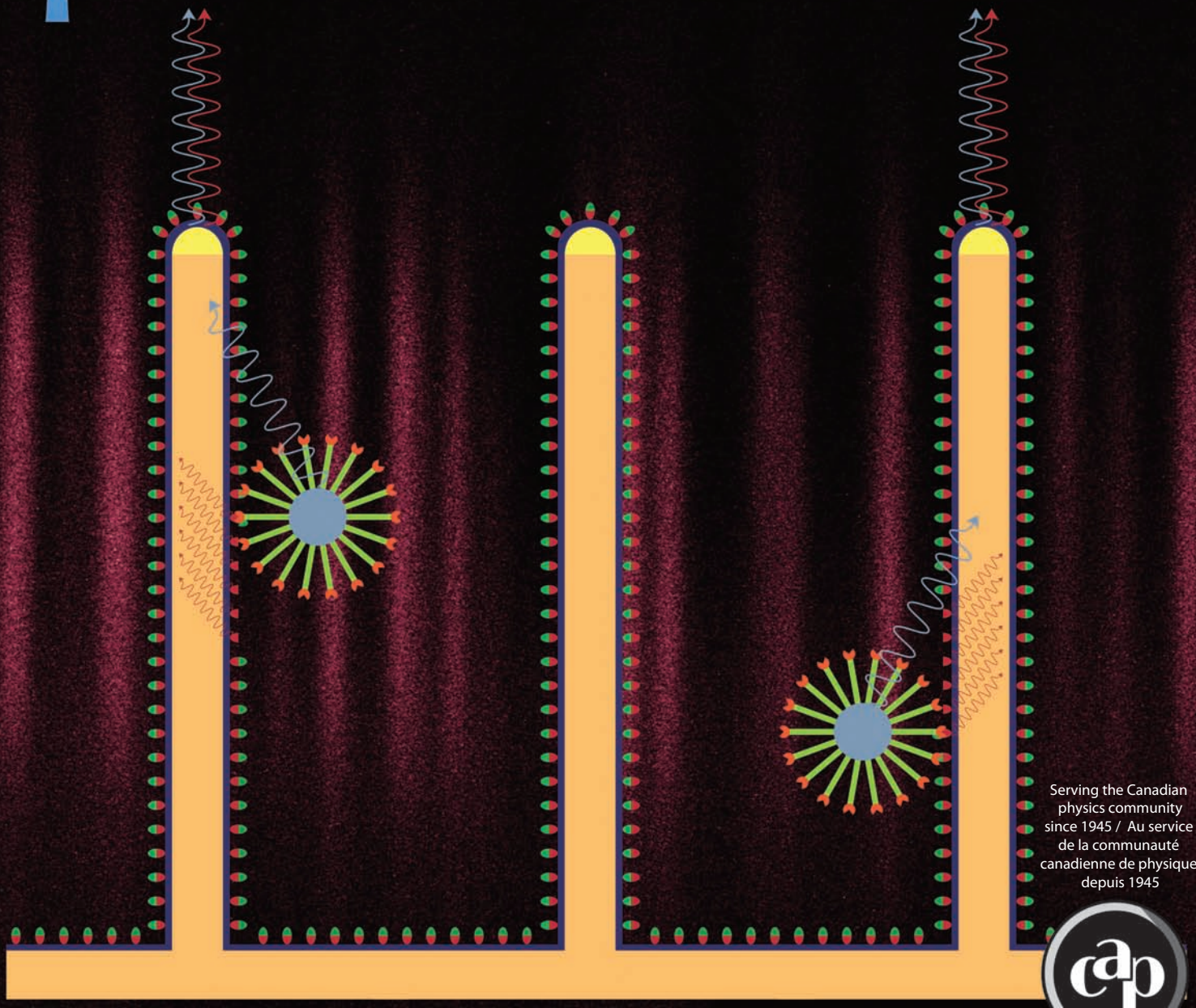

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# Nanoscale Approaches to Biological Systems / *Systèmes biologiques : approches nanoscopiques*

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# Physics in Canada
# La Physique au Canada

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**Cover / Couverture :**


Schematic of synthetic "Lawnmower" molecular motors, cutting fluorogenic peptide lawns coupled to light-guiding nanowires. Background shows fluorescent side image of a nanowire array. Images courtesy Damiano Verdaro and Heiner Linke, Lund University, Sweden. See article by Korosec and Forde in this issue.

*Schéma de moteurs moléculaires synthétiques appelés "tondeuses à gazon" qui coupent des gazons de peptides fluorogéniques couplés à des nanofils optiques. L'arrière-plan montre le côté fluorescent d'un faisceau de nanofils. Images courtoisie de Damiano Verdaro et Heiner Linke, Université Lund, Suède. Voir l'article de Korosec et Forde dans ce numéro.*

## Physics Education Articles



## Departments / Départements



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The Canadian Association of Physicists was founded in 1945 as a non-profit association representing the interests of Canadian physicists. The CAP is a broadly-based national network of physicists working in Canadian educational, industrial, and research settings. We are a strong and effective advocacy group for support of, and excellence in, physics research and education. We represent the voice of Canadian physicists to government, granting agencies, and many international scientific societies. We are an enthusiastic sponsor of events and activities promoting Canadian physics and physicists, including the CAP's annual congress and national physics journal. We are proud to offer and continually enhance our web site as a key resource for individuals pursuing careers in physics and physics education. Details of the many activities of the Association can be found at http://www.cap.ca. Membership application forms are also available in the membership section of that website.

L'Association canadienne des physiciens et physiciennes a été fondée en 1946 comme une association à but non-lucratif représentant les intérêts des physicien(ne)s canadien(ne)s. L'ACP est un vaste regroupement de physiciens oeuvrant dans les milieux canadiens de l'éducation, de l'industrie et de la recherche. Nous constituons un groupe de pression solide et efficace, ayant pour objectif le soutien de la recherche et de l'éducation en physique, et leur excellence. Nous sommes le porte-parole des physiciens canadiens face au gouvernement, aux organismes subventionnaires et à plusieurs sociétés scientifiques internationales. Nous nous faisons le promoteur enthousiaste d'événements et d'activités mettant à l'avant-scène la physique et les physiciens canadiens, en particulier le congrès annuel et la revue de l'Association. Nous sommes fiers d'offrir et de développer continuellement notre site Web pour en faire une ressource clé pour ceux qui poursuivent leur carrière en physique et dans l'enseignement de la physique. Vous pouvez trouver les renseignements concernant les nombreuses activités de l'ACP à http://www.cap.ca. Les formulaires d'adhésion sont aussi disponibles dans la rubrique «Adhésion» sur ce site.

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# NANOPHYSICAL APPROACHES TO BIOLOGICAL SYSTEMS

In perusing this special issue of *Physics in Canada*, you may wonder *"what is biology doing in a physics journal?"* To answer this question, one must realize that the field of biology is undergoing a revolution in which emergent collective behaviours of *molecules* take center stage. A major thrust of twenty-first century physics is to achieve a deep understanding of the form and function of these molecular collectives and to address fundamental questions such as *"what is life?"*. These challenges run as deep as those about the origin of the universe, and are as fundamental as those about the indistinguishable nature of quantum particles.

While biological and biochemical assays have traditionally been used to identify biomolecules and binding between biomolecules, theoretical and experimental tools from the domain of *nanoscale physics* can provide new insights into our understanding of the structure, dynamics and self-assembly of biological molecules and systems. In the wet, squishy, and highly fluctuating interior of the living cell, entropic confinement plays a key role in determining molecular motions and shapes. The precise and robust nature of cell division can be understood using the languages of out-of-equilibrium statistical mechanics and phase-transition theory. The stochastic playgrounds of biological molecules are qualitatively estranged from the land of Newton's cannonballs and inclined planes and Carnot's heat engines, and are typically missing from introductory physics curricula.

We hope that this issue reveals how the field of biological physics is rich with new paths in statistical mechanics, polymer physics, and soft matter. Pioneering work in these areas of physics depends on multiple perspectives offered by physical, biological and health scientists. These multidisciplinary collaborations integrate directly with the diagnostics, biotechnology and pharmaceutical industries.

This issue features articles from just a small subset of the excellent Canadian researchers applying nanoscale physics to biological systems. Some of the articles address questions of understanding this nanoscale world in terms of thermodynamic concepts: the importance of entropy and confinement (Sabrina Leslie and colleagues); mapping free energy surfaces of unfolding proteins and nucleic acids (Michael Woodside); and developing theoretical tools and experimental approaches to understanding the operation of nanoscale machines (Aidan Brown and David Sivak, and Chapin Korosec and Nancy Forde, respectively). Others demonstrate how new imaging technologies are pushing our understanding of molecular dynamics and organization within cells (Joshua Milstein and colleagues) and are providing insight into nanoscale mechanics of assembled protein materials (Laurent Kreplak and colleagues). Finally, three articles explore the transition of nanoscale biophysics from the research laboratory to "real-world" applications: the use of solid-state nanopores for sequencing nucleotides (Vincent Tabbard-Cossa and colleagues); the discovery, development and commercialization of a unique plant-based biomolecule for personal care and biomedical applications (John Dutcher and colleagues); and advice on commercializing research technology in Canada (Andre Marziali).

The message of this issue, especially to the youngest and most curious physicists into whose hands this issue has fallen, is that there is *plenty of work to be done by physicists – theoretical, experimental and technological – to get to the bottom of life's mysteries*. There is an abundance of fascinating physical principles and tools to explore, develop, and use in the context of open biological questions and open biotechnology challenges. Squeezing, pulling and probing single molecules can create paradigm shifts of understanding, which can open up new directions in biotechnology, a thrust which is supported by the growing Canadian entrepreneurial scene. To educators, we hope that the research and education articles in this issue will lead to more focus in our classrooms and laboratories on these exciting new areas of physics research.

We invite you to read the articles in this issue written by Canadian leaders in different areas of biological physics. We hope that they will inspire and inform the next generation of scientists and technologists, and help you to see how nanoscale physics can be used to advance our understanding of the richness and complexities of life.

Sincerely,

John Dutcher, Nancy Forde, Sabrina Leslie
Guest Editors

*Comments from readers on this Foreword are more than welcome.*

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# SYSTÈMES BIOLOGIQUES : APPROCHES NANOPHYSIQUES

La lecture de ce numéro spécial de *La Physique au Canada* vous amènera à vous demander : *pourquoi parler de biologie dans une revue de physique?* Pour répondre à cette question, il faut se rendre compte que le domaine de la biologie subit une révolution qui donne un rôle central aux nouveaux comportements collectifs des molécules. Un axe majeur de la biologie du 21e siècle est de faire bien comprendre la forme et la fonction de ces comportements collectifs et de répondre à des questions fondamentales telles « *qu'est-ce que la vie?* ». Ces enjeux sont aussi cruciaux que ceux entourant l'origine de l'Univers et aussi fondamentaux que ceux touchant la nature indiscernable des particules quantiques.

Les essais biologiques et biochimiques servent depuis toujours à identifier les biomolécules et la liaison entre elles, mais les outils théoriques et expérimentaux du domaine de la *physique à l'échelle nanométrique* peuvent permettre de mieux comprendre la structure, la dynamique et l'autoassemblage des molécules et systèmes biologiques. Dans l'intérieur humide, souple et très changeant de la cellule vivante, le confinement entropique joue un rôle clé pour ce qui est de déterminer les mouvements et formes moléculaires. La nature précise et robuste de la division cellulaire peut être comprise grâce aux langages de la mécanique statistique à l'équilibre rompu et de la théorie de la transition de phase. Les aires stochastiques des molécules biologiques sont séparées qualitativement de celle des boulets de canon et plans inclinés de Newton, et des moteurs thermiques de Carnot, et sont généralement absentes des programmes d'introduction à la physique.

Nous espérons que ce numéro révèle à quel point la physique biologique ouvre de nouveaux domaines en mécanique statistique, en physique des polymères et en matière molle. Les travaux novateurs dans ces domaines de la physique dépendent des perspectives multiples offertes par les spécialistes en physique, biologie et santé. Ces collaborations multidisciplinaires intègrent directement les industries diagnostique, biotechnologique et pharmaceutique.

Ce numéro présente des articles de seulement un petit sous-ensemble d'excellents chercheurs canadiens qui appliquent la physique nanométrique aux systèmes biologiques. Certains articles traitent de questions de compréhension de ce monde nanométrique sur le plan des concepts thermodynamiques : l'importance de l'entropie et du confinement (Sabrina Leslie et collègues); le mappage des surfaces d'énergie libre des protéines et acides nucléiques qui se déploient (Michael Woodside); et la conception des outils théoriques et des approches expérimentales à la compréhension du fonctionnement des machines nanométriques (Aidan Brown et David Sivak, et Chapin Korosec et Nancy Forde, respectivement). D'autres démontrent comment de nouvelles technologies d'imagerie améliorent notre compréhension de la dynamique et de l'organisation des molécules au sein des cellules (Joshua Milstein et collègues) et permettent de mieux comprendre les mécanismes nanométriques des matériaux protéiques assemblés (Laurent Kreplak et collègues). Enfin, trois articles explorent la transition de la biophysique nanométrique du laboratoire de recherche vers des applications « réelles » : l'utilisation de nanopores à l'état solide pour le séquençage de nucléotides (Vincent Tabbard-Cossa et collègues); la découverte, l'élaboration et la commercialisation d'une biomolécule unique à base de plantes pour les soins personnels et des applications biomédicales (John Dutcher et collègues); et un avis sur la commercialisation de la technologie de la recherche au Canada (Andre Marziali).

Le message de ce numéro, notamment pour les physiciens les plus jeunes et les plus curieux qui en ont pris connaissance, est que pour *les physiciens – théoriques, expérimentaux et technologiques – il y a bien du travail à faire pour percer les mystères de la vie*. Il y a de très nombreux et fascinants principes et outils à explorer, élaborer et utiliser en physique dans le contexte des questions biologiques et enjeux biotechnologiques non résolus. Comprimer, tirer et sonder de simples molécules peut entraîner des changements de paradigme qui, dans la compréhension, peuvent susciter de nouvelles orientations en biotechnologie, axe qui est appuyé par la scène entrepreneuriale canadienne en pleine croissance. Pour les enseignants, nous espérons que les articles de ce numéro sur la recherche et l'éducation contribueront à mieux orienter les classes et laboratoires vers ces nouveaux domaines passionnants de la recherche en physique.

Nous vous invitons à lire les articles de ce numéro, signés par des chefs de file canadiens dans divers domaines de la physique biologique. Nous espérons qu'ils sauront inspirer et informer la prochaine génération de scientifiques et de technologues et vous aideront à discerner comment la physique nanométrique peut faire avancer notre compréhension de la richesse et des complexités de la vie.

Cordiales salutations.

John Dutcher, Nancy Forde, Sabrina Leslie
Rédacteurs honoraires

*Les commentaires de nos lecteurs (ou) lectrices au sujet de cette préface sont les bienvenus.*

*NOTE : Le genre masculin n'a été utilisé que pour alléger le texte.*

# TOWARD THE DESIGN PRINCIPLES OF MOLECULAR MACHINES

BY AIDAN I. BROWN AND DAVID A. SIVAK

**SUMMARY**

**Living things avoid equilibrium using molecular machines. Such microscopic soft-matter objects encounter relatively large friction and fluctuations. We discuss design principles for effective molecular machine operation in this unfamiliar context.**

### Life is Fundamentally Out of Equilibrium

From the largest mammals down to unicellular organisms, living things manifest orderly structures, processes, and flows that are inconsistent with a state of thermal equilibrium [1]. Cells, the micron-scale structural units of life, maintain out-of-equilibrium conditions of chemical concentrations, charge and molecular distributions, and unequal pressures [2,3]. This ubiquity of out-of-equilibrium states flies in the face of the Second Law of thermodynamics, which pushes towards increased entropy in the absence of coordinated free energy input.

### Life Stays Out of Equilibrium Using Molecular Machines

Cells rely heavily on many types of molecular machines—macromolecular complexes that convert between different forms of energy—to achieve various tasks that create and maintain low-entropy structure. The molecular machine adenosine triphosphate (ATP) synthase [4] is noteworthy, as it couples to nonequilibrium electrochemical distributions to drive the otherwise free-energetically unfavourable synthesis of ATP. Many other machines then couple to the nonequilibrium ratio of ATP to adenosine diphosphate (ADP) to drive other unfavourable reactions. *E.g.*, transport motors (such as kinesin [5] and myosin [6]) distribute cargoes to make them less uniform or to beat the natural timescales of diffusion. These naturally evolved machines provide inspiration for the design of synthetic molecular machines, including those that walk [7], rotate [8], and pump [9] (for a discussion of natural and synthetic molecular motors, see this issue's article by Chapin Korosec and Nancy Forde).

A system at thermal equilibrium cannot have nonzero net (average) flux between different states. For example, in Feynman's ratchet [10], a wheel with asymmetric teeth engages with a pawl to prevent clockwise rotation. When coupled to a windmill, occasionally enough gas molecules hit the windmill to turn the wheel in the permitted counterclockwise direction. This directed mechanical work, rectifying thermal energy, appears to violate the Second Law. However, thermal fluctuations sufficient to turn the wheel are also sufficient to disengage the pawl, allowing clockwise rotation [11]. The thermal fluctuations produce no net motion on average, hence the Second Law is preserved.

Hence molecular machines at equilibrium are not functional and do no useful work: they are as likely to hydrolyze ATP as synthesize it; as likely to transport cargoes to the left as to the right. These machines both maintain nonequilibrium conditions and themselves operate far from equilibrium, paying a free energy cost to escape thermodynamic equilibrium and achieve directed behaviour.

## NANOSCALE MACHINES RUN DIFFERENTLY

Molecular machines and their components are nanometer-sized, and such tiny objects interact with their environment in ways that defy our physical intuition honed with macroscopic, human-sized objects.

### Friction and Inertia

The balance of inertial and viscous (frictional) forces is quantified by the dimensionless Reynolds number $Re \equiv vL/(\mu/\rho)$, for velocity $v$, characteristic linear dimension $L$, dynamic viscosity $\mu$, and mass density $\rho$. The micron-sized bacterium *E. coli* (diameter ~ 1μm) can swim up to 40 μm/s [12], which in room-temperature (20°C) water corresponds to $Re \sim 10^{-5}$. Viscous forces thus completely dominate its inertia [13], so much so that if it were to immediately stop actively swimming, it would coast only ~10 pm, less than $10^{-5}$ of its own size.

Nanometer-sized molecular machines have even lower Reynolds number and less inertia. The average motion of such nanoscale objects persists only as long as

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something continues to 'push:' they rapidly 'forget' how fast and in what direction they were traveling, producing overdamped behaviour. Unlike a car motor, a molecular machine cannot rely on inertia to carry it to its next stage of operation.

### Fluctuations

Although low Reynolds number corresponds to overdamped motion, nanoscale objects do not require external driving forces to move. On the contrary, these nanometer-sized objects, composed of relatively soft protein material, have energy scales comparable to the thermal energy $k_BT$ present at room temperature, so stochastic fluctuations of motion are omnipresent. Similar to the diffusive behavior of pollen grains in water [14], the components of molecular machines are constantly jostled by collisions with the surrounding medium (typically water or other proteins). Even a driven molecular machine will move in a given direction only on average, with frequent pauses and back-steps. Single-molecule experiments now directly observe this stochastic motion [15].

This overdamped motion and strong stochastic fluctuations suggest that nonequilibrium and statistical approaches are central to understanding the behaviour and design of molecular machines.

## NONEQUILIBRIUM STATISTICAL MECHANICS / STOCHASTIC THERMODYNAMICS

### Driven Processes

To study a system's behaviour out of equilibrium, we can experimentally push on it. The equilibrium ensemble can be parameterized by a control parameter $\lambda$, a knob that an experimentalist can turn to change the system. Common experimental control parameters for probing molecular machines include: the distance between foci of optical traps or between an atomic force microscope (AFM) cantilever and an immobile surface, or the rotational angle of a magnetic trap. A protocol $\Lambda$ specifies a schedule for changing the control parameter from initial value $\lambda_i$ to final $\lambda_f$. For example, one can increase the distance between the foci of optical traps, thereby unfolding a biomacromolecule stretched between them (see this issue's article by Michael Woodside).

### The Second Law at Microscopic Scales

To understand the generic nonequilibrium behaviour of stochastic systems (including molecular machines), we begin with a familiar result of macroscopic physics. One form of the Second Law of thermodynamics states that the work $W$ required to drive a system via control protocol $\Lambda$ between two control parameter values and hence two corresponding equilibrium ensembles,

$$W[\Lambda] \geq \Delta F_\Lambda, \tag{1}$$

exceeds the equilibrium free energy change $\Delta F_\Lambda$ between the beginning and end of the protocol $\Lambda$. Microscopic systems, with few degrees of freedom, behave with a significant stochastic component: any particular microscopic realization may 'violate' the Second Law, using less work than the free energy change. In this light, we realize that the Second Law is really about averages,

$$\langle W \rangle_\Lambda \geq \Delta F_\Lambda, \tag{2}$$

with the angled brackets $\langle \cdot \rangle$ representing an ensemble average over many stochastic system responses to the protocol $\Lambda$. What we now recognize as the macroscopic version of the Second Law [Eq. (1)] holds for systems with Avogadro's number of degrees of freedom, where statistical fluctuations are negligible.

Research in recent decades has uncovered what are, in effect, statistical generalizations of the Second Law to microscopic systems. The Jarzynski relation [16] places a tight constraint on nonequilibrium response through an equality between the (exponentiated) free energy change and the exponential average of the work,

$$\langle e^{-\beta W} \rangle_\Lambda = e^{-\beta \Delta F_\Lambda}, \tag{3}$$

for $\beta \equiv (k_BT)^{-1}$, Boltzmann's constant $k_B$, and environmental temperature $T$. The statistical Second Law [Eq. (2)] is immediately recovered upon applying Jensen's inequality [17], which states that for a convex function $f$ and random variable $Y$, $\langle f(Y) \rangle \geq f(\langle Y \rangle)$.

### Statistics of Microscopic Reversibility

In systems satisfying microscopic reversibility [18], for any forward trajectory the time-reversed trajectory is also possible. A further generalization of the Second Law under conditions of microscopic reversibility is the Crooks fluctuation theorem [19, 20],

$$\frac{P_\Lambda(X)}{P_{\tilde{\Lambda}}(\tilde{X})} = \exp[\beta(W[X|\Lambda] - \Delta F_\Lambda)], \tag{4}$$

where $P_\Lambda(X)$ is the probability that the system follows trajectory $X$ during the forward protocol $\Lambda$, $P_{\tilde{\Lambda}}(\tilde{X})$ the probability for the time-reversed trajectory $\tilde{X}$ during the reverse protocol $\tilde{\Lambda}$, and $W[X|\Lambda]$ is the work used to drive the system along trajectory $X$ during the forward protocol. This places an even stronger constraint on nonequilibrium behaviour: *for each trajectory* there is a fixed ratio between the probability of a forward trajectory in response to the forward protocol, and the probability of the time-reversed trajectory for the reverse protocol. Rearranging and averaging over trajectories, given a particular protocol, recovers the Jarzynski relation.

These so-called fluctuation theorems relate reversibility to work and free energy changes. More general nonequilibrium contexts feature multiple distinct nonequilibrium drives beyond an external work source: multiple reservoirs of temperature and/or chemical potential, out-of-equilibrium boundary conditions such as shearing, etc. For such systems, the sufficient statistic governing reversibility is entropy production (in the system and environment) [21]. Here the more general form of the Crooks theorem is

$$\frac{P_\Lambda(X)}{P_{\tilde{\Lambda}}(\tilde{X})} = \exp\left(\frac{\Delta S[X]}{k_B}\right), \tag{5}$$

with entropy production $\Delta S[X]$ during the forward trajectory $X$.

Recent research seeks to apply these fundamental theoretical advances in the context of biomolecular energy and information conversion. What limits are imposed on core biological processes by basic physical considerations? What design principles describe systems that reach those limits? Are these principles manifested in machines evolved by nature? And do they give useful insights for designing novel machines?

## OPTIMAL CONTROL

Just as we judge the efficiency of a car engine compared to theoretical limits (the Carnot limit or finite power equivalents), how do evolved and synthetic molecular machine efficiencies compare with the physical limits for stochastic nonequilibrium energy transduction? More abstractly, how can one drive a system from one equilibrium ensemble to another, in a fixed timespan, with the least energetic effort (work)?

### Theory

For a control parameter changing sufficiently slowly, the required instantaneous input power is [22]

$$P(t_0) = \left(\frac{dF}{d\lambda}\frac{d\lambda}{dt}\right)_{t=t_0} + \zeta(\lambda(t_0)) \cdot \left(\frac{d\lambda}{dt}\right)^2_{t=t_0}. \tag{6}$$

The first term represents the equilibrium free energy change, the work done in the quasistatic limit when the system remains equilibrated throughout the protocol. The second term represents the excess power, the extra energy required due to the system being out of equilibrium, and it depends on a generalized friction coefficient $\zeta$ in the space of control parameters.

$\zeta$ governs near-equilibrium response and represents the energetic cost of changing the control parameter sufficiently fast to drive the system out of equilibrium. It is a function of equilibrium system fluctuations,

$$\zeta(\lambda(t_0)) \equiv \beta \int_0^\infty dt \langle \delta f(0) \delta f(t) \rangle_{\lambda(t_0)}, \tag{7}$$

where $\delta f \equiv f(t_0) - \langle f \rangle_{\lambda(t_0)}$ is the deviation of the conjugate force $f$ from its equilibrium average $\langle f \rangle_{\lambda(t_0)}$ at fixed control parameter value $\lambda(t_0)$. For instance, when the control parameter is the optical trap position, the conjugate force is the tensile force with which the biomolecule resists further extension. $\langle \delta f(0) \delta f(t) \rangle_{\lambda(t_0)}$ is an autocorrelation function: at $t = 0$ it equals the force variance, while for $t > 0$ the autocorrelation function represents how quickly the system forgets its initial condition. Equation (7) is an example of a fluctuation-dissipation theorem relating equilibrium fluctuations to dissipation out of equilibrium.

How do we minimize this extra energy to waste as little as possible? In Eq. (6), the friction coefficient [Eq. (7)] expresses the rate of excess work accumulation along a control protocol – this friction coefficient defines a metric providing a measure of path length in control parameter space. Protocols that minimize dissipation follow geodesics (shortest paths) in the curved space defined by the friction coefficient metric. This is entirely analogous to minimization of airline flight distances along great circle routes: though on a Mercator projector these routes look curved, they are in fact shortest paths on the curved surface of the earth.

Along such optimal paths, the optimal protocol proceeds such that the excess power is constant:

$$\frac{d\lambda^{opt}}{dt} \propto \frac{1}{\sqrt{\zeta(\lambda(t))}}. \tag{8}$$

We have used this framework to examine optimal protocols in model systems [22-25].

### Ramifications for ATP Synthase

ATP synthase couples transport of hydrogen ions down their gradient to synthesis of ATP from ADP and phosphate, against a chemical potential difference favouring ATP hydrolysis. Though ATP synthase is a large and intricate molecular complex, communication is mediated through a relatively simple mechanical coordinate, the rotational angle of a crankshaft connecting the integral membrane $F_o$ subunit to the soluble $F_1$ subunit (Fig. 1). Single-molecule studies of ATP synthase typically excise the $F_o$ subunit, attach an experimental handle (e.g., a magnetic bead) to the crankshaft, and monitor or force rotation using a magnetic trap. Such experiments suggest that $F_1$ can approach near 100% efficiency [31,32].

Experimental observation of $F_1$ rotational statistics indicates a small number of metastable angular states separated by energetic barriers. When the magnetic trap is centered at an energetic barrier, equilibrium probability is equally split

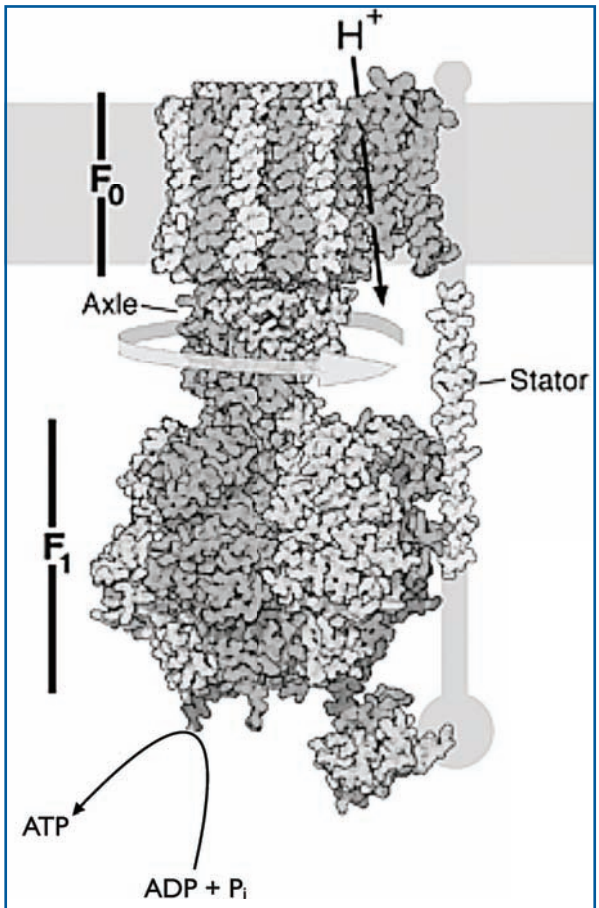


Fig. 1 ATP synthase, a composite rotary motor. The top motor, $F_o$, is embedded in the membrane and couples proton flow across the membrane (down its electrochemical gradient) to rotation of the central axle or crankshaft. The bottom motor, $F_1$, couples this rotation to synthesis of ATP from ADP and phosphate. Image adapted from the Protein Data Bank [26-30].

between the adjacent metastable states, giving maximal force variance $\langle \delta f^2 \rangle$ and maximal force relaxation time, hence maximizing their product, the friction coefficient. Equation (8) provides intuition on how an experimentalist (or $F_o$ *in vivo*) should drive rotation to minimize energy expenditure: where the friction coefficient is large—where the system puts up large resistance to rapid control parameter changes, at the rotational energetic barriers—the minimum-dissipation protocol proceeds slowly, giving thermal fluctuations maximal time to kick the system over the barrier 'for free.' For a double-well potential, a protocol optimized according to Eq. (8) can dissipate less than half the energy of a naive (constant-velocity) protocol [25].

## NONEQUILIBRIUM FREE ENERGY

In general, driving a system out of equilibrium costs energy, beyond the system's equilibrium free energy change $\Delta F^{\mathrm{eq}}$. What happens to this extra energy put into the system, and how much of it can be used to do useful work?

The system free energy, in or out of equilibrium [33], can be defined as $F = \langle E \rangle - TS$, with average energy $\langle E \rangle = \sum_x P(x)E(x)$and entropy $S = -k_{\mathrm{B}} \sum_x P(x) \ln P(x)$, for system microstates $x$. At the conclusion of a nonequilibrium protocol $\Lambda$, the system will generally be out of equilibrium. The final nonequilibrium distribution $P_\Lambda^{\mathrm{neq}}(x)$ over microstates will generally have a larger free energy $F_\Lambda^{\mathrm{neq}}$ than the equilibrium ensemble $P_\lambda^{\mathrm{eq}}(x)$ corresponding to the final control parameter value $\lambda$. As the system relaxes to equilibrium from this nonequilibrium state, it can do work on a coupled mechanical system. The available work is the difference between these nonequilibrium and equilibrium free energies,

$$F_\Lambda^{\mathrm{neq}} - F_\lambda^{\mathrm{eq}} = k_{\mathrm{B}} T \, D(P_\Lambda^{\mathrm{neq}} \| P_\lambda^{\mathrm{eq}}), \tag{9}$$

which is proportional to a central information-theoretic quantity, the relative entropy (Kullback-Leibler divergence) [17] $D(P_1 \| P_2) \equiv \sum_x P_1 \ln[P_1(x)/P_2(x)]$ between the nonequilibrium and equilibrium probability distributions. Full probability distributions require vast numbers of samples to measure experimentally, but using the Crooks theorem this relative entropy can be estimated from measurements of mean work [33].

Molecular machines couple nonequilibrium reactions, and thus as part of their core function may utilize this nonequilibrium free energy, say by driving a downstream process using the transiently available free energy following activation by an upstream process.

## TRADEOFFS OF DISSIPATION AND TIME SYMMETRY BREAKING

Eliminating energy waste cannot be the only goal – a perfectly efficient process (dissipating no energy) would be completely reversible, and hence unable to achieve directed behavior. The Second Law says that irreversible operation requires *some* energy loss, but is silent about quantitative details.

Irreversibility, which can be thought of as the 'length of time's arrow' [34], can be operationalized through the intuitive question: if I reverse the flow of time, how different will my dynamics look? More quantitatively, how different is the ensemble of time-reversed trajectories from the more familiar forward-time trajectory ensemble? The Jensen-Shannon divergence $J$ [17] between these two trajectory ensembles quantifies the average information gain, upon observing a trajectory randomly drawn with equal probability from either ensemble, about which ensemble it comes from [35]. Essentially, this quantity tells us how weird a movie of a system's dynamics looks when played backwards.

Sufficiently slow processes remain near equilibrium, and provide a universal near-equilibrium result for irreversibility for a given amount of energy dissipation [34]. For a simple model of energy storage, irreversibility can be significantly increased above the near-equilibrium result by coupling storage of moderate-sized energy packets with moderate mechanical motion [36], suggesting the energy and length scales of molecular machines may be constrained by a requirement for forward progress [37].

## FUTURE PROSPECTS

### Stochastic Driving

Much of the theory above focuses on deterministic driving of a system, where a control parameter follows a fixed temporal schedule. Biomolecular machines do not typically experience an experimentalist deterministically changing a control parameter – instead they operate autonomously, responding to the stochastic fluctuations of coupled nonequilibrium systems. Recent research [38,39] has opened new vistas on the physical principles governing autonomous molecular machines driven by stochastic protocols.

### Fundamental Understanding of Constraints for Biology

Although molecular machines are built and operate inside living cells, they are subject to physical constraints. In this article, we have outlined how molecular machines operate out of equilibrium to overcome viscous forces in the face of fluctuations. We also describe recent developments for efficient system control and generation of irreversible dynamics. This emerging understanding of the statistical physics of driven microscopic processes points toward general principles for molecular machine operation.

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# NANOMECHANICS OF PROTEIN FILAMENTS

BY SAMUEL J. BALDWIN, ANDREW S. QUIGLEY, AND LAURENT KREPLAK

One of the hallmarks of mammalian tissues is the presence of several interconnected filamentous protein networks both within each cell, the so-called cytoskeleton [1], and within the extracellular matrix [2]. Each network is a dynamic assembly of protein filaments and associated molecules that can sustain mechanical stresses as well as be remodeled in response to the same. Considering the importance of filamentous networks in maintaining the structural integrity of tissues, it comes as no surprise that their mechanical properties have attracted a lot of attention both experimentally and theoretically [3,4]. At the network level, most studies so far ignore all the molecular level diversity found in protein filaments and treat them as slender rods with a combination of entropic and enthalpic elasticity [5]. This simple approach has been very successful in identifying common features in the mechanical properties of a wide range of networks built of actin filaments, intermediate filaments, fibrin fibers and collagen fibrils, for example. It does not mean however that these different networks are interchangeable; in fact each type of protein filament has evolved to achieve a defined set of physical and biological functions. This is why measuring the mechanical properties of single protein filaments is also of great interest even if it is to some extent more challenging than dealing with macroscopic networks.

Any method developed to test the mechanical properties of single protein filaments has to contend with three issues: the filaments are small with diameters between 1 and 100s of nanometers, the forces necessary to bend or stretch them are anywhere in the pico-Newton to micro-Newton range, and the measurements have to be repeated many times to be representative of a large population. Currently there are many approaches available from micropipette manipulation [6] to optical tweezers [7], microelectromechanical systems (MEMS) [8], stretchable substrates [9], and atomic force microscopy (AFM) [10].

**SUMMARY**

**We discuss atomic force microscopy based approaches to study the structural and mechanical properties of protein filaments using intermediate filaments and collagen fibrils as examples.**

As an example we will discuss a group of AFM-based approaches that have proven successful in testing cytoskeletal filaments and collagen fibrils.

The AFM has three main modes of operations: imaging, force spectroscopy or indentation, and manipulation (Fig. 1a) [11]. Each of these can provide useful mechanical information on single filaments attached to a solid support in a liquid environment.

## NANOMECHANICS OF CYTOSKELETAL FILAMENTS

The mammalian cell's cytoskeleton is composed of three types of protein filaments: actin filaments, intermediate filaments such as keratin, vimentin and lamin, and microtubules [1]. For each of these filaments, the three main mechanical quantities of interest are the degree of bending due to thermal fluctuations, the Young's and shear moduli, and their ultimate tensile properties, typically their maximum extensibility and their ultimate tensile strength.

### Imaging flexible filaments on a surface

In first approximation cytoskeletal protein filaments are not different from long, flexible, polymer chains. Single protein filaments in solution at constant temperature experience shape fluctuations that can be characterized using the worm-like chain model by a persistence length P [12]. In turn, P provides an estimate of the bending rigidity of the filament assuming it is a uniform, homogenous, elastic material [12]. For cytoskeletal filaments the shape fluctuations occur over sub-micrometer to millimeter length scales depending on the protein and can be measured by fluorescence microscopy [13]. Another approach is to let the filaments attach to a flat substrate and image their "frozen" contour by AFM [12]. For this method to work the filaments must be dilute enough on the substrate to avoid any overlap with neighbors, they must appear flexible on the scale of their contour length and they must have reached their equilibrium conformation on the substrate. The last point is typically difficult to assess and requires testing different buffer conditions or substrates with different surface chemistry. In the case of cytoskeletal filaments it is often not feasible to change buffer conditions because they have a strong impact on the assembly state of the filaments. For example divalent cations like calcium or

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magnesium tend to bundle actin [14] and intermediate filaments [15]. Varying buffer and substrates for three different types of intermediate filaments demonstrated a wide range of morphologies from twisted bundles to beaded filaments and thin tapes [16]. For vimentin intermediate filaments in standard assembly conditions, we investigated mica, graphite and glass, and obtained a persistence length of 1 micrometer [12]. Assuming these filaments are uniform homogeneous elastic cylinders with a diameter of 10 nm, we get an equivalent Young's modulus of 8 MPa. This number describes the flexibility of vimentin filaments that are much longer than their persistence length, 1 micrometer. What happens if we test these filaments on a length scale smaller than their persistence length?

### Bending intermediate filaments

The most direct way to measure the bending stiffness of a beam is a two-point or three-point bending test. For microtubules that have a persistence length in the millimeter range, the two types of tests were performed using optical tracking [17] and AFM imaging [18], respectively. With the optical method, it was possible to demonstrate that bending of microtubules is in fact length dependent [17]. With AFM imaging of microtubules absorbed on a membrane with manufactured slits of varying width around 100 nm, it was possible to estimate the Young's and shear moduli using elasticity theory [18]. In principle if the microtubules were just cylinders filled with an isotropic material, the ratio of Young's modulus to shear modulus is typically smaller than 3, however the experimentally measured ratio is around 1000 indicating that microtubules are anisotropic.

For vimentin intermediate filaments we used a similar AFM approach using porous alumina membranes with a pore size of 250 nm [19]. The apparent bending modulus in this geometry was 300 MPa, which puts a lower bound on the filaments Young's modulus assuming no shear deformation [19]. There is then a contradiction between the persistence length measurements discussed above and this three point bending result. One way to resolve it is to consider the possibility of shear deformations within the vimentin intermediate filaments. Interestingly, vimentin intermediate filaments are built by the lateral packing of staggered double stranded alpha-helical coiled-coils which are slender 45 nm long rods with a diameter around 2 nm [20]. The Young's modulus of a single coiled-coil is in the order of 1 GPa [21]. Assuming this is also the Young's modulus of a vimentin intermediate filament, the three-point bending test yields a shear modulus of 2.5 MPa. As expected the vimentin intermediate filament is strongly anisotropic and the Young's modulus estimated from the persistence length data appear to be on the order of the shear modulus. In other words thermal fluctuations of the filament's shape are mostly due to a shear deformation mode at the molecular level.

### Stretching intermediate filaments

So far we have only discussed the mechanical properties of intermediate filaments in the small deformation limit. To access the tensile properties of these filaments, one can take advantage of the manipulation capabilities of the AFM. Intermediate filaments are absorbed on a flat substrate in liquid and the AFM probe, pressed against the surface, is moved on a path perpendicular to the filament's axis (Fig. 1a) [1]. For the right AFM cantilever stiffness, one observes that the filament is locally bent and stretched by the probe until it breaks. AFM imaging after manipulation reveals that 100 to 500 nm long segments of the filaments were extended by 50 to 250% strain and that the diameter of the filaments decreased from 10-12 nm down to 2 nm for the largest strains (Fig. 1b) [22]. Considering that the maximum extensibility of an alpha-helical coiled-coil is around 150% strain [23], these results confirm that two molecular mechanisms are at play during elongation, unfolding of the coiled coils and sliding of these units past each other. Furthermore, the AFM offers the possibility to measure the force applied to the filament during manipulation. Forces to break a single intermediate filament are between 1 and 5 nN [24,25]. The force-displacement data can also be fit using a two-state model in order to extract the unfolding force of a single coiled-coil unit, which is around 10 pN for desmin intermediate filaments [26].

## NANOMECHANICS OF COLLAGEN FIBRILS

Collagen fibrils are another ubiquitous protein filament within the mammalian kingdom. These fibrils have diameters between 50 and 500 nm. They form densely packed networks in skin, tendons, ligaments, cartilage and bone and act as a mechanical scaffold for cells within these tissues. Similarly to intermediate filaments, collagen fibrils are linear aggregates of staggered rod-shaped collagen molecules 300 nm long and 1.5 nm in diameter [27]. However collagen fibrils also have specific-covalent crosslinks between molecules that further stabilize the axial staggering pattern and that are essential for the fibrils load bearing functions within tissues [28]. At the single fibril level, two main AFM derived approaches are commonly used to assess mechanical properties: nanoindentation and tensile testing.

### Probing collagen fibrils' molecular architecture via nanoscale indentation

To perform a nanoindentation experiment, the AFM probe is pressed into a single collagen fibril until a target deflection of the cantilever is achieved. Knowing the tip-sample geometry, one can fit the force-displacement curve and extract the indentation modulus, which is around 1-5 MPa [10] for a hydrated fibril or 1-5 GPa [29] for a dried fibril at low indentation speeds. Due to the geometry of the nanoidentation experiment and the anisotropic nature of the collagen fibrils, the indentation modulus is not an intrinsic mechanical constant such as the Young's modulus; rather it measures the degree of lateral cohesion between collagen molecules within the fibril.

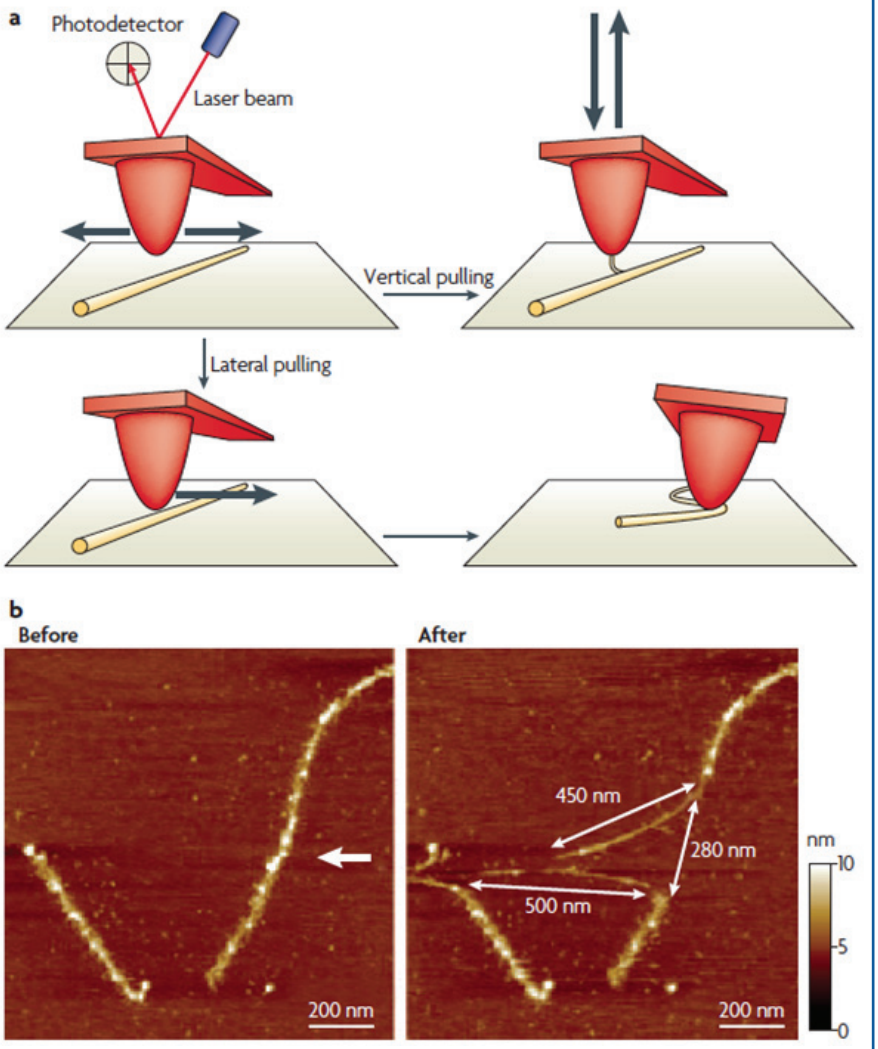


Fig. 1 a) Sketch of the three basic modes of operations of an atomic force microscope (AFM), imaging, force spectroscopy and manipulation. b) AFM image of a neurofilament (a type of intermediate filament found in the brain), before and after manipulation by the probe moving from right to left along the white arrow. A 280 nm long neurofilament segment was stretched into two thin branches 950 nm long in total [1].

As such the indentation modulus should be sensitive to the density and connectivity of crosslinks, and to the density of collagen molecules in the fibril cross-section. Interestingly the axial stagger of the molecules within the fibril is expected to generate regular gaps between the molecules that produce a periodic fluctuation in molecular density with a characteristic length scale of 67 nm [30]. The gap regions of the fibrils are expected to have 4/5 the molecular density of the overlap regions, and this provides a density contrast over a length scale that can be easily resolved using a sharp AFM probe. However, nano-indentation measurements along single dried collagen fibril revealed a factor of two in indentation modulus between gap and overlap regions [29]. Similar measurements were carried along hydrated collagen fibrils in various buffer conditions but revealed no contrast in indentation modulus between gap and overlap regions [10]. In order to understand the lack of contrast along hydrated collagen fibrils we explored the impact of the tip velocity on the indentation modulus. We observed that for tip velocities between 0.1 and 100 μm/s, the indentation modulus of a collagen fibril in water is proportional to the logarithm of the velocity [27] as expected for rubbers above the glass transition temperature [31]. Above 100 μm/s the indentation modulus increased by a factor of 3 to 5 over one decade [27]. This sharp increase in indentation modulus is also observed for rubbers below the glass transition temperature and is the signature of a characteristic relaxation time of the tip-fibril interaction. As long as the indentation occurs over a time scale longer than the relaxation time, viscous effects dominate and the indentation modulus is insensitive to contrasts in molecular density. When the indentation occurs over a time scale shorter than the relaxation time, viscous modes of deformation are "frozen out", the fibril appears stiffer than before and one expects the indentation modulus to be sensitive to how densely packed the molecules are in the vicinity of the probe. This assumption was confirmed by performing nanoindentation maps along single collagen fibrils in water with a tip velocity of 600 μm/s. We observed that the indentation modulus of the gap regions was 80% of the one measured in the overlap regions, confirming the molecular density prediction (Fig. 2a and c) [27]. These experiments served as a proof of principle to study the effect of plastic deformation, generated by mechanical overload, on the fibrils structure. We were then able to demonstrate that fibrils extracted from overloaded bovine tail tendons have a core with preserved molecular packing surrounded by a loose and disordered shell (Fig. 2b and d) [32]. In order to establish the relationship between stress, strain and structural damage, we need to move away from tensile testing of full tendons where loading is not homogeneous to tensile experiments at the single fibril level.

**Tensile testing of single collagen fibrils**

So far there has been two ways of measuring the stress-strain curve of a single collagen fibril: using an AFM in the force

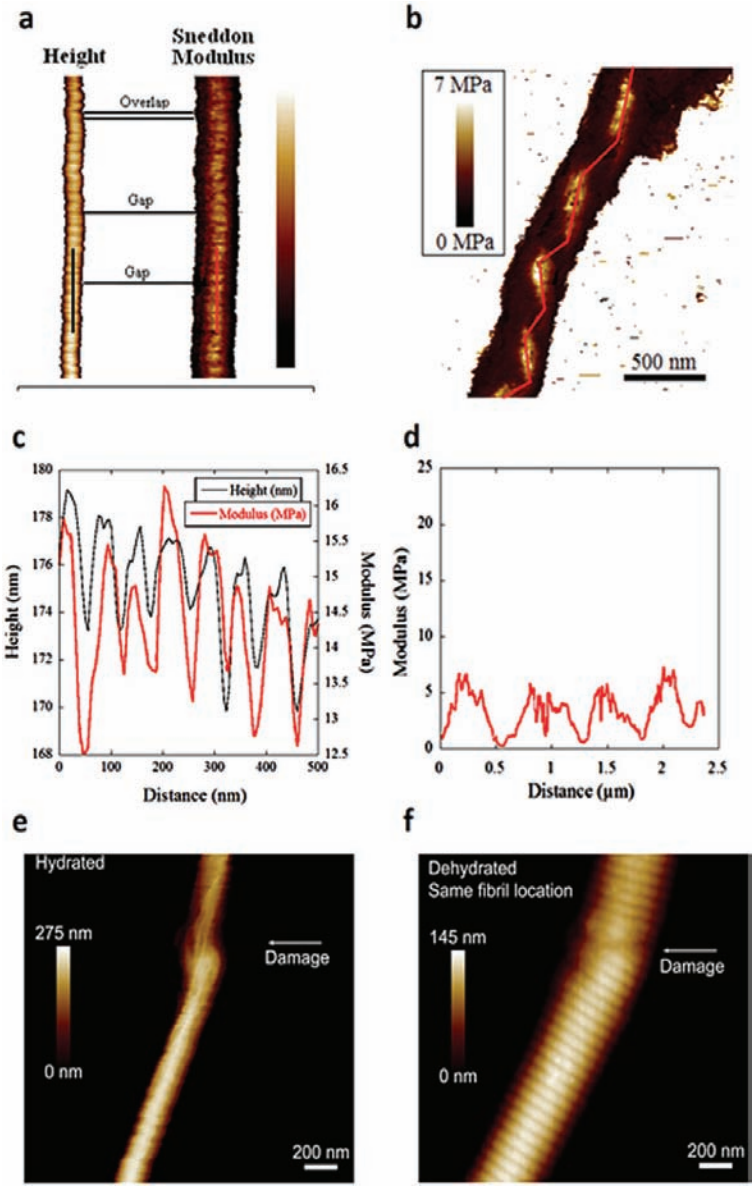


Fig. 2 a) Matched height and indentation modulus data obtained for a hydrated collagen fibril extracted from rat tail, the bar represents 2 μm (adapted from [27]). b) Indentation modulus map of a hydrated collagen fibril extracted from a cyclically overloaded bovine tail tendon (adapted from [32]). c) Matched height and indentation modulus profiles taken along the apex of the fibril in a). Note that the periodic gap and overlap fluctuations in height, the overlap being always higher than the gap, are matched with similar fluctuations in modulus (adapted from [27]). d) Indentation modulus profile taken along the apex of the overloaded fibril in b) (adapted from [32]). Notice the periodic gap and overlap fluctuation in c) compared to the micrometer scale fluctuation in d). The decrease in indentation modulus from c) to d) is due to the presence of a disordered shell surrounding a more compact core. e) and f) hydrated and dehydrated images of the same fibril after tensile failure (adapted from [33]). Notice the striking difference of morphology at the damaged site.

spectroscopy mode [34] and using microelectromechanical systems (MEMS) [8]. A collagen fibril typically breaks between 20 and 50% strain depending on its length and pulling speed. The force necessary to reach failure is in the 1-10 μN range. Fibrils broken using these two approaches show either no damage except at the ruptured ends or lateral molecular packing disruption similar to the core-shell morphology described above. For both methods, it is not easy to study the effect of loading without rupture on the fibril structure. To circumvent this issue, we have developed an in-plane stretching technique inspired from previous nanomanipulation studies on intermediate filaments (Fig. 1b) [33]. Fibrils are dried on a substrate to allow laying down thin strips of glue at regular intervals. This procedure generates isolated fibril segments that can be rehydrated and manipulated by moving an AFM probe perpendicular to the segment's long axis, stretching the fibril like a bowstring. The entire manipulation process can be recorded with a video microscope as long as the fibrils have a diameter above 100 nm, which is the case for most fibrils extracted from mature tendons. After loading, the fibrils remain on the substrate and can be imaged in air or in liquid (Fig. 2e and f). Using this approach we observed that the indentation modulus of fibrils stretched to 3-4% and released dropped by a factor of 2, while the hydrated height of the fibrils increased by 30% [33]. Subsequent stretching of the same pre-conditioned fibrils to strains up to 20% without rupture yielded a decrease in indentation modulus by a up to a factor of 5 while the height of the fibrils randomly increased by 10 to 70% without a clear strain dependence [33]. Furthermore, some of the fibrils ruptured during the pulling process, which gave us an opportunity to observe the vast morphological differences between the hydrated and dehydrated state of a damaged fibril (Fig. 2e and f) [33]. In the future we intend to also record the force necessary to pull the fibrils and correlate the observed changes in fibril architecture with stress-strain data.

## CONCLUSION

It is clear from the few examples highlighted here that we are only beginning to understand the complexity arising from the self-assembly of proteins into filaments. Measuring mechanical properties at the single filament level is a

promising way forward in this field, as it will allow researchers to dissect the impact of small changes at the protein level on the properties of the filamentous assembly. However it should also be complemented by structural measurements at the single filament level through, for example, nanoscale vibrational spectroscopy techniques [35,36].

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# BIOLOGICAL CONFINEMENT PHYSICS: SQUEEZING NEW INFORMATION OUT OF COMPLEX MACROMOLECULES

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By confining and visualizing biomolecules on molecular to microscopic dimensions, we can directly investigate how confinement physics can regulate macromolecular behaviour. New tools enable us to tackle open biological questions on how physiologically important molecules, such as chromosomal DNA or proteins, behave in nanoscale environments such as the nucleus or the ribosome respectively. Despite strong interest, getting into this "*room at the bottom*" has presented challenges to existing tools. Here, we review a suite of single-molecule methods and experiments that we have devised to study how biomolecules respond to incrementally smaller spaces of applied confinement, as well as their own topology, emulating naturally occuring nano confinement structures; both behaviour and topology can be controlled continuously, and with facility by our methods. The overarching vision of our research is to investigate the complex behaviour of biomolecules, such as protein, DNA, and RNA, with respect to the principle of applied confinement.

## INTRODUCTION

Why is the development of new approaches to visualize and manipulate *the behavior of single biomolecules*, *on molecular to microscopic dimensions*, so important to the frontiers of research in biological physics, biotechnology, biology, biochemistry, and nanoscience? It is for two principal reasons: firstly, biomolecules must function in wet, squishy, and crowded cellular environments [1,2]; and secondly, "next-generation" genomics and proteomics technologies can leverage new insights and techniques to advance biomedicine [3]. To this effect, theoretical physicist Henrik Flyvbjerg wrote a commentary in *P.N.A.S.* on our pioneering efforts in single-molecule technology, which he called "*Getting into that room at the bottom*" [4]. Dr. Flyvberg was referring to Nobel Laureate Richard Feynman's visionary 1959 lecture, entitled "*There's plenty of room at the bottom*", in which Feynman predicted a world of invention and discovery of tiny machines and devices, on the order of a billionth of a meter in size, the so-called "nano world". Today, many of these inventions have fundamentally changed our lives, ranging from new nanomaterials to miniature biomedical sensors, which have improved medical treatments, and have provided a growing context for pioneering single-molecule technologies. Dr. Flyvbjerg describes the unique nano manipulation and imaging tools that we are developing as an *important solution to the general problem of controlling and understanding biomolecules "at the bottom" of the length scale*, from Angstroms to micrometers; that is, being able to continuously visualize and hence establish the functional mechanisms of the "nano world", at the intellectual frontiers of materials science, engineering, biology and biomedicine.

Building on this context, this *P.i.C* article reviews our recent contributions to "getting to the bottom" of understanding DNA molecules – the complex biopolymers which encode our genetic makeup – from three perspectives: as a coiled polymer with fascinating physical properties under nano confinement, relevant to understanding the dense interior of the cellular nucleus; as a sequence of bases which stores our genetic code, relevant to understanding evolution and disease; and as a dynamic, structural scaffold which regulates essential cellular processes, relevant to understanding DNA replication, repair and gene transcription.

In line with these overarching perspectives, we review our single-molecule investigations of how DNA conformations and dynamics become qualitatively different when squeezed into nanoscale spaces. Building on this fundamental understanding, we apply the tools we are creating to contribute to the development of a "next generation" of "single-molecule biomedicine" – where the properties of individual protein or DNA molecules can be critical to understanding and detecting disease states. We explore how continuously adjusting the applied confinement

**SUMMARY**

**We study how confinement physics regulates macromolecular behaviour, from nano to microscopic dimensions. New tools enable us to tackle open questions on how physiologically important biopolymers behave in nanoscale environments.**

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enables us to literally "unscroll" tangled "DNA-blob" conformations into more regular "DNA-rod" conformations, which are easier to interrogate. By developing gentle, high-throughput approaches to visualizing and manipulating macromolecules, we can contribute not only to biological confinement physics, but also to the advancement of biomedicine.

## METHOD

We design and build *de-novo* instrumentation to acquire continuous and high-quality movies of the dynamics and shapes of biomolecules, on adjustable nanoscale dimensions. Building upon our published principle of *Convex Lens-induced Confinement* (CLiC) imaging [5,6], we have developed a microfluidic imaging tool, which mounts on a regular inverted microscope, transforming it into a single-molecule manipulation and visualization station [7]. For example, by confining long, linear DNA molecules within ring-shaped traps (Fig. 1d), we can dramatically enhance the efficiency of their "self-ligation" into loops.

### Why is "CLiC imaging" important to advancing biophysics?

The state-of-the-art of detecting macromolecular assembly and dynamics primarily comprises methods to watch single molecules bound to each other, to surfaces created by other macromolecules, or to polymers of the cellular cytoskeleton such as DNA or microtubules, respectively. Commonly used methods, such as "total internal reflection fluorescence" (TIRF) microscopy, allow detection of the appearance and disappearance of individual molecules on or near to a surface. What CLiC provides that TIRF does not allow is the capacity to continuously follow the trajectory of a freely diffusing single molecule for long periods, before it is bound to a substrate, when it is bound and when it dissociates. This capability is particularly important in biophysics because many elements of cellular activity, such as DNA transcription or movement of molecules on microtubules are processive; that is, individual events are dependent on previous events. This memory can often be inferred, but not directly measured with current techniques; but while tracking dynamics with CLiC it now becomes possible to measure. Thus, it is possible that CLiC will contribute to verifying or disproving some of the most fundamental models of biomolecular dynamics, such as DNA-topology-mediated regulation of transcription. Furthermore, by looking at molecules in a thin volume, compared to other techniques, CLiC microscopy can resolve single-molecule trajectories in the presence of orders of magnitude higher reagent concentrations, and over orders of magnitude longer periods, allowing us to discern and follow weak and slow interactions – which is relevant to a wide variety of physiological processes.

For example, in an ongoing collaboration with biochemist Dr. David Levens (NIH Cancer Center) and mathematician Dr. Craig Benham (UC Davis Genome Center), we are studying how a topological property of DNA called "supercoiling" influences the interactions between specific probe molecules and specific unwinding sites on the supercoiled DNA. By trapping the molecules using CLiC, and

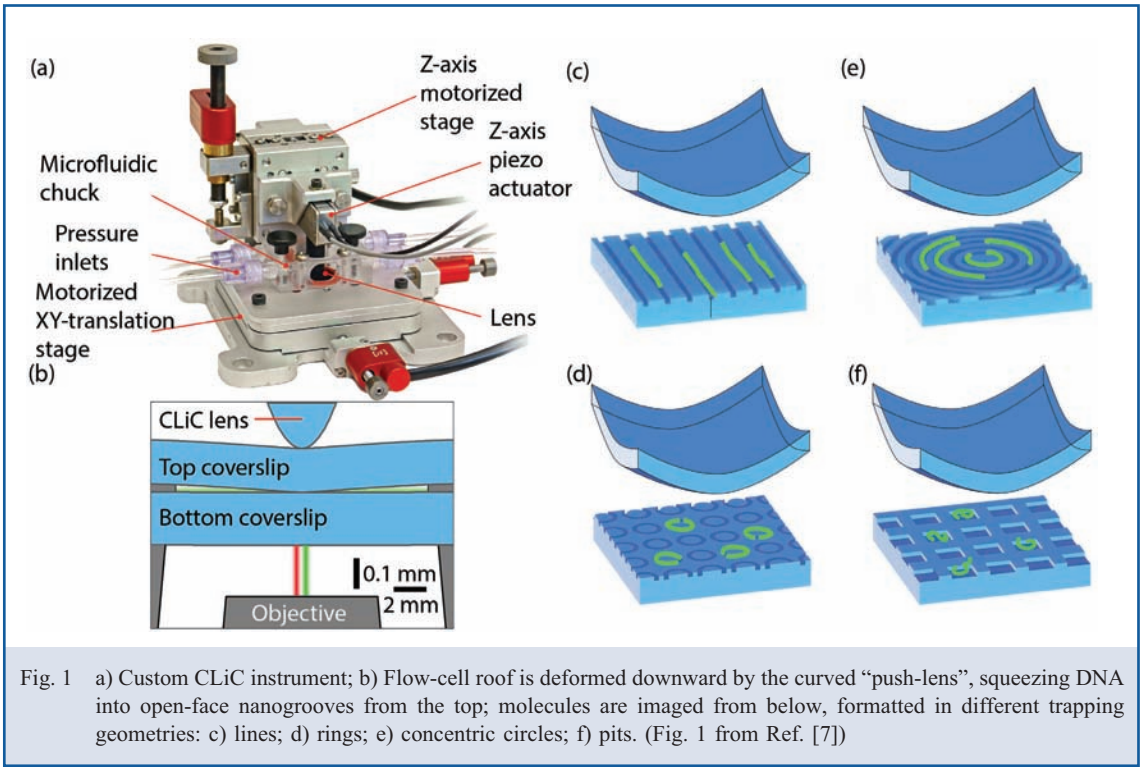


Fig. 1 a) Custom CLiC instrument; b) Flow-cell roof is deformed downward by the curved "push-lens", squeezing DNA into open-face nanogrooves from the top; molecules are imaged from below, formatted in different trapping geometries: c) lines; d) rings; e) concentric circles; f) pits. (Fig. 1 from Ref. [7])

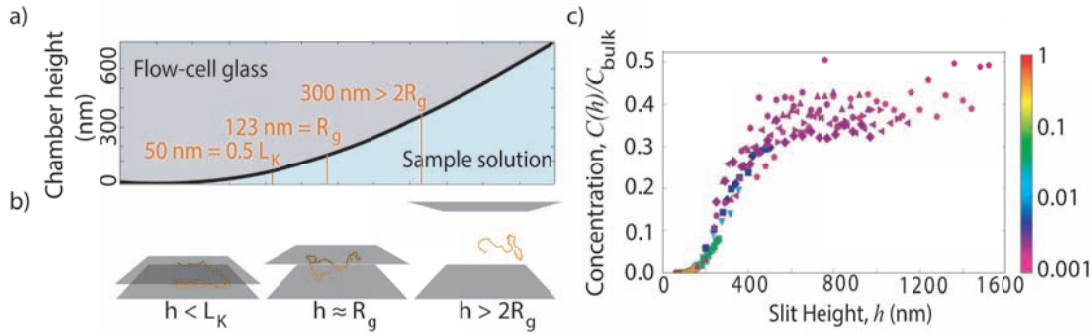


Fig. 2 a) Schematic of a static CLiC imaging chamber (where the flow-cell has been deformed by the push-lens). b) Renderings of different polymer conformations in different confinement regimes obtained by simulation. c) Scaled concentration of the DNA molecules as a function of the confinement height *h*. (Fig. 2 from Ref. [8])

following molecular interaction trajectories over thousands of seconds, we can determine how the binding/unbinding kinetics are influenced by the DNA topology. These insights are important to testing models of gene regulation at a single-molecule level, which were previously inaccessible with other methods under "untethered" molecular conditions.

## RESULTS

### We are exploring transitions in polymer conformations on nanoscale dimensions

CLiC technology allows us to perform rigorous equilibrium studies of transitions in DNA conformations, e.g., from "blob-like" in an unconfined regime, to "rod-like" in a nanoconfined regime (Fig. 2) [8]. Importantly, we can visualize DNA over several orders of magnitude of applied confinement, in a single experiment and device.

Our research contributes a direct measurement of the "free energy of confinement" experienced by the DNA molecules, over several orders of magnitude of applied confinement. When a flexible polymer is confined, fewer conformations are accessible, making confinement energetically unfavourable. Our measurement of the confinement free energy is direct: we count the number of DNA molecules, at equilibrium, in a single chamber of smoothly varying height. A much lower density of molecules is observed in regions of the chamber where the height is much smaller than the diameter of unconstrained molecules (Fig. 2c).

Importantly, our data provide a "bridge" that connects prior measurements and theories in the literature, typically obtained in limiting cases using devices of fixed dimensions. Our experimental data and simulations have served to link a), "nano confinement theory" (by Odijk [9]), b), an interpolation model which extends Odijk to "moderate confinement" (by Chen and Sullivan [10]) and c), "unconfined theory" which describes the transition from moderate to unconfined conditions (by Casassa [11]). In the nanoconfined regime, the applied confinement is so severe that the polymer is unable to form random coils the way it does in free solution; its conformations look like "deflecting stiff segments" off of the walls. In the unconfined regime, the polymer appears as a "blob", as one would intuitively expect. In between – in moderate confinement – the DNA conformations smoothly transition from a deflecting rod, to a network of blobs, to a single blob, according to theory that our experiments and simulations agree with.

### We can dynamically manipulate DNA conformations with *in-situ* visualization

Here, we leverage our fundamental understanding of, and physical control over DNA conformations, to study the *dynamic manipulation* of DNA molecules [4]. We use the curved surface of the "push-lens" (Fig. 1a) to dynamically deform the top flexible coverslip above the bottom coverslip, which contains the embedded nanogrooves, during the CLiC imaging experiment. This work establishes a novel method for *entropic trapping and dynamic stretching* of DNA molecules in grooves, which is compatible with single-molecule, "long-read" genomic analysis. In particular, we can extend DNA in sub 50-nm nano-channels with high and uniform stretching (90%), in agreement with the Odijk deflection theory, and useful towards "long-read" sequence measurements [12,13].

### How is nanoscale DNA confinement physics relevant to advancing genomics?

Modern medicine has entered the genomic age; the promise of genomic medicine is to customize optimal treatments for individuals and genetically similar people. Human chromosomes are between 25 million and 425 million bases long; however today's commonly used, high-accuracy DNA sequencing techniques can generally read DNA molecules that are 250 bases long or shorter. Typically, they break chromosomes into random, short and overlapping fragments,

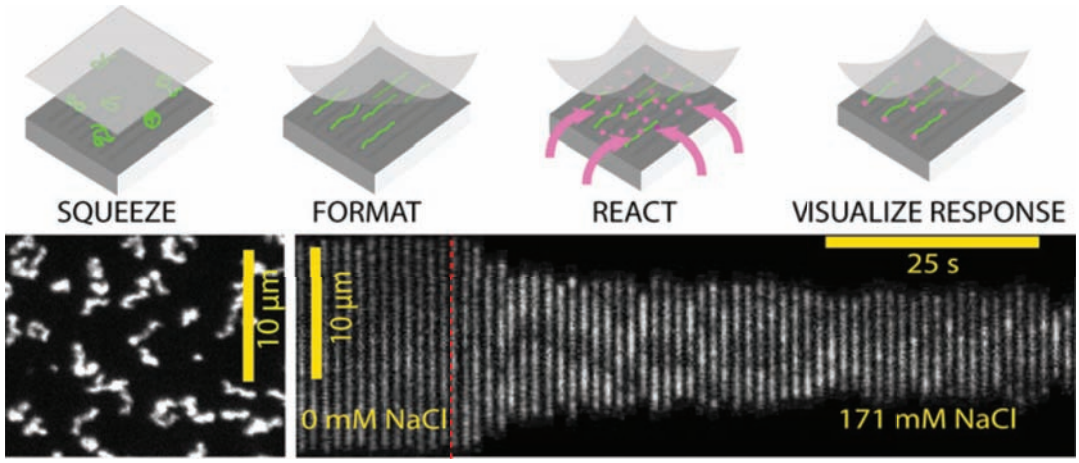


Fig. 3 (Top) Schematic process of squeezing and linearizing DNA molecules into nanogrooves, reaction with reagents, and visualizing the DNA response. (Bottom, left) Image of DNA being squeezed; (right) Kymograph of a single DNA in a nanogroove, whose extension is decreasing over time, due to the exchange of the buffer with a higher salt solution. ("Thumbnail" of Ref. [14])

sequence them, and then match overlapping sequences to each other to reassemble the entire sequence, like pieces of a puzzle. While computational approaches can put more than half of the puzzle back together, this has not worked for critical regions of the human genome containing repeats of the same sequences, such as the genes that code for antibodies, the molecules that protect us from invading bacteria and viruses. To contribute to the growing field of "long-read" genomics, we are continuing to advance our understanding of, and control over DNA in nano-confined spaces, while miniaturizing our tools into a "technology-compatible" format.

### We can control and visualize biophysical chemistry in nano confined reactors

Our recent work has demonstrated a new approach to 1) trapping and formatting the shapes of DNA within custom-shaped (e.g., linear or circular) embedded grooves; and 2) introducing reagents and visualizing interactions, without disrupting the molecules [14]. For instance, we can enhance the "joining" of the ends of linear DNA, which are confined in circular nanogrooves, to form closed circular DNA loops, by introducing "ligase enzymes" into the chamber from the side. During these processes the DNA conformations are only limited by the walls of their containers; that is, not tethered.

## OUTLOOK

### A "tether-free", single-molecule playground for squeezing and visualizing macromolecules, on nanoscale dimensions

Acquiring continuous movies of the dynamic change in biomolecular properties, in response to biochemical and physical variables, enables direct investigation of important biophysical mechanisms. In collaboration with biologists, biochemists, chemists and biophysicists, we aim to apply CLiC technology to investigate a myriad of confinement-mediated processes in the near term, such as protein condensation, protein folding, membrane biophysics and DNA nanostructure dynamics. With the advancement of sophisticated nanolithography tools running in parallel with our research, we are excited by the promise of carving increasingly complex physiological architectures out of glass and silicon walls; allowing us to isolate, watch and understand complex biological molecules at work, in an ever-expanding *room at the bottom*.

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# ENGINEERING NANOSCALE BIOLOGICAL MOLECULAR MOTORS

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In his book 'What is life?', Erwin Schrödinger equated death with the living cell decaying into thermal equilibrium with its surroundings. That is, when a cell is unable to continue using the free energy of its environment to keep order, it decays into a state of disorder. Evolution has led to incredibly beautiful, complex, and astonishing constructs called molecular motors to maintain order and sustain life.

Molecular motors are tiny, nanometer-sized, protein-based machines often described as the 'workhorses' of the cell. They are involved in a wide range of key processes, including intracellular transport, positioning of cell organelles, cell division, muscle contraction, and synthesis of ATP, the chemical 'fuel' of the cell (Table 1).

In an effort to understand the complex operation of biological molecular motors, researchers have sought to create synthetic ones. The importance of this approach, a young and growing area of research, was recognized by this year's Nobel Prize in Chemistry. Following the view of Richard Feynman, "What I cannot create, I do not understand" [1], we test our understanding of mechanisms of operation through designing artificial analogues of the processes we observe in Nature.

In this article we highlight some properties of biological molecular motors, then focus on the relatively new field of biologically inspired *artificial* molecular motors.

**SUMMARY**

**Understanding the operation of biological molecular motors, nanoscale machines that transduce electrochemical energy into mechanical work, is enhanced by bottom-up strategies to synthesize novel motors.**

## KINESIN: A PROTOTYPICAL WALKING MOTOR

A well known family of biological motors is kinesin. The motor protein kinesin I functions primarily to transport large cargo that will not randomly diffuse in a reasonable time scale to its needed place in the cytoplasm. It does so by walking in a hand-over-hand fashion directionally along a microtubule 'track' within the cell. Kinesin I consists of two flexibly linked globular head groups (the 'feet') each with 2 binding sites: one site for ATP and the other for the microtubule surface (Fig. 1: top). When kinesin's leading head is bound to a microtubule, ATP can bind into the active site. The neck linker then stiffens such that the lagging head is propelled forward to become the new leading head [2]. Random thermal fluctuations ultimately bring the leading head to dock to the next microtubule binding site. The ATP in the lagging head is hydrolyzed to ADP + phosphate. Rapid diffusion of the phosphate ion into solution results in a sub-nanometre 'gap' within the ATP active site [3]. It is believed that this triggers a conformational reorganization near the active site that is mechanically transmitted to the microtubule binding site. This lagging motor head then unbinds from the track, and the cycle repeats itself (Fig. 1: bottom). Interestingly, there is evidence that Kinesin I remains in an idle position until a cargo binds [4]; the binding of cargo activates the motor to begin moving!

Kinesin by the numbers: Kinesin I takes 8 nm steps at an average velocity of 750 nm/s, and is able to exert a force of up to 5.4 pN [5]. Given that kinesin's mass is 184 kDa (where 1 kDa = $1.66 \times 10^{-9}$ picograms), its ratio of maximum force to body weight is over $10^9$. If a person were capable of such a force-to-body weight ratio they would be able to lift more than 40000 Boeing 747 aircraft. The incredible difference between the relative capabilities of kinesin versus people arises because molecular systems operate in a stochastic environment where they harness energy in the form of thermal fluctuations. (For a detailed discussion on this topic see the article by David Sivak and Aidan Brown in this issue.)

## SYNTHETIC WALKING MOTORS

Inspired in part by kinesin, DNA-based nanomachines have shown great promise as artificial molecular motors. Here, there are two sources of energy that can be used to bias motion: hydrogen bonding associated with DNA basepairing, and hydrolysis (cleavage) of DNA's phosphodiester backbone [6]. The free energy change of creating 10 base pairs of DNA is approximately $-58$ kJ mol$^{-1}$ ($-23$ $k_BT$ at 300K), similar to that of

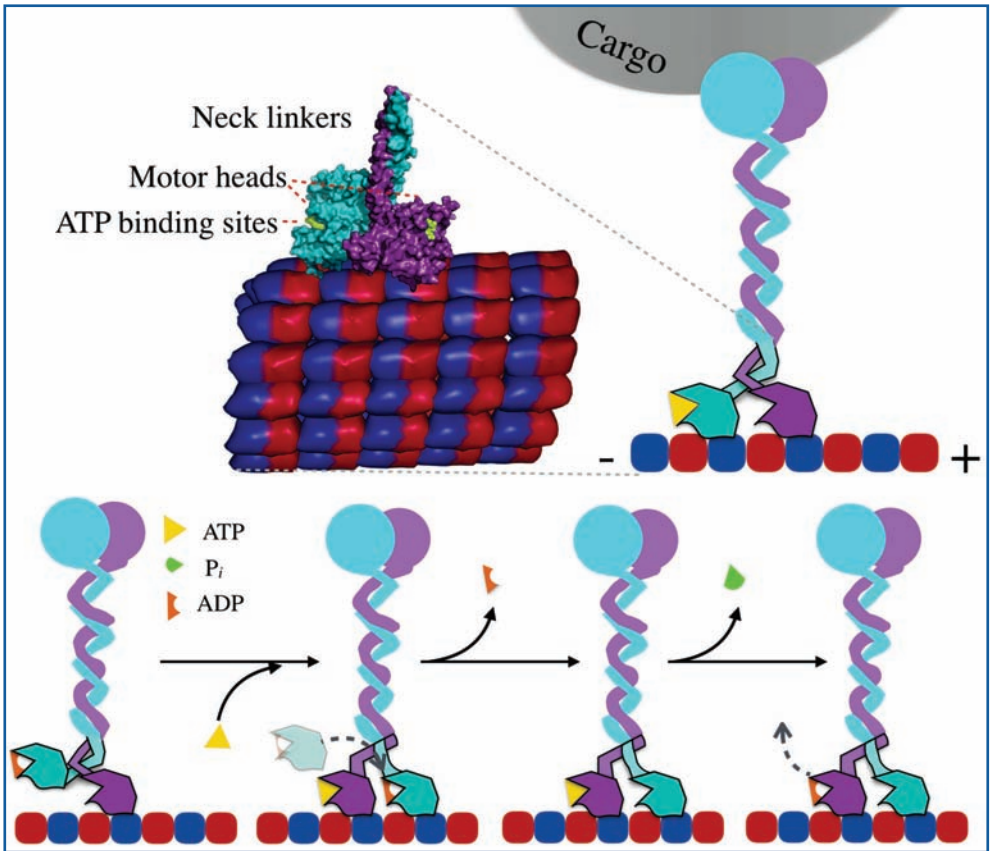


Fig. 1 Kinesin crystal structure and walking schematic. Top: crystal structure of kinesin I motor heads (Protein Data Bank (PDB) 1N6M) and neck linkers docked onto a microtubule (PDB 3JAO). Kinesin motor heads bind tubulin (the single protein building units of microtubules) and walk towards the plus end of the microtubule (towards the cell periphery). Bottom: A motor head in the empty state binds the microtubule, with the lagging head behind. The neck linker stiffens in response to ATP binding, which drives the lagging head forward towards the next binding location. ATP in the now lagging head is hydrolysed to ADP and phosphate ($P_i$), which results in the detachment of this head from the track.The cycle continues with each ATP hydrolysed corresponding to one step forward.

ATP hydrolysis at standard conditions, $-32$ kJ mol$^{-1}$ ($-13$ $k_BT$ at 300K)[6]. For reference, the thermal energy scale of $k_BT$ is 2.5 kJ/mol (or $\sim$0.03 eV) at 300 K. As an example of a DNA nanomachine, Tosan Omabegho and co-workers [7] successfully constructed a bipedal DNA walker that relies on diffusion to move directionally on an asymmetric track. Its hand-over-hand translocation (between DNA footholds supported on a double-stranded DNA track) is analogous to the motility mechanism of kinesin. Its anticipated speed is, however, much slower than that of kinesin due to the timescales of DNA hybridization and unbinding. This DNA nanomachine is one of the few autonomous artificial molecular motors thus far realized.

Another approach to artificial design, much more in its infancy, utilizes non-motor-protein modules as building blocks. The 'tumbleweed'[8] is an example of a tripedal motor designed to move on a periodically patterned, linear dsDNA track. Its feet are three unique DNA-binding protein domains each chemically linked to a central hub. By flowing through the appropriate ligand for each foot [9], the construct is expected to take successive steps along a DNA track in a tumbling fashion, as shown in Fig. 2. Similar to most DNA motors, the tumbleweed cannot operate autonomously, but relies on rectified diffusion, controlled by cyclically flowing in the correct activators for each foot. For this design, the free energy source for biasing movement is a temporal change in chemical potential: the high concentration of new ligand drives a specific foot to bind, and when that ligand is removed from solution the low concentration leads to the release of the foot.

## BURNT-BRIDGES RATCHETS: BIOLOGICAL AND SYNTHETIC

Another class of motile proteins in biology achieves directional and processive motion through a 'burnt-bridges' mechanism. A burnt-bridges walker destroys track binding sites as it progresses, thereby biasing its own motion by preventing backwards stepping.

Matrix metalloproteases (MMPs) are an example of such a system [10]. MMPs travel along collagen fibrils, degrading these tracks (catalysing the cleavage and subsequent disassembly of collagen) as they travel. (For more information on collagen fibrils, see the article by Baldwin, Quigley and Kreplak in this issue.) Observations of individual MMP-1 proteins have characterized their motion as biased one-dimensional diffusion, biased by substrate cleavage and hindered by specific interactions with the collagen fibrillar track [11]. Intriguingly, the cleavage sites for MMP are separated by 65 nm along the collagen fibril, a distance approximately an order of magnitude larger than the size of the MMP. There are many open questions regarding how MMPs achieve biased directionality and an extremely high processivity. Recent studies suggest that it is not sufficient to examine simply the MMP, but rather one must

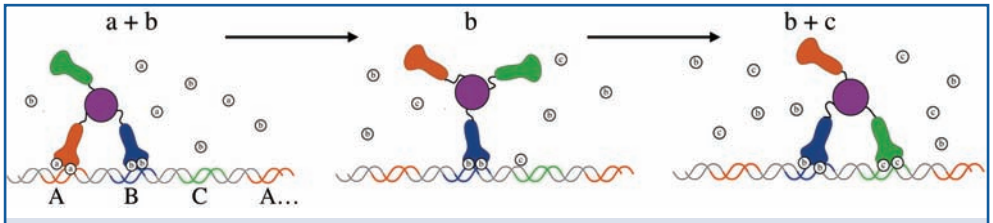


Fig. 2 Protein-based design of the tumbleweed. Figure adapted from ref. [8]. The tumbleweed motor is comprised of three chemically linked DNA-binding proteins. The DNA track is patterned with binding sequences specific for each protein on the tumbleweed motor. The presence of the corresponding ligand for each foot facilitates its binding. From left to right: The tumbleweed begins with the *A* and *B* feet bound in the presence of ligands *a* and *b*. When the solution is replaced by one containing only ligand *b*, the resulting lower concentration of ligand *a* leads to dissociation of ligand *a* from foot *A* resulting in the release of the foot from the track. Ligands *b* and *c* are then flowed in, which promotes the additional binding of foot *C*. The process continues cyclically to step the motor along the one-dimensional DNA track.

consider the collagen fibrillar track and its structure as an integral part of the motility mechanism [12].

An artificial DNA motor that utilizes hydrolysis to induce a burnt-bridges mechanism has been constructed by Bath *et al.* [6]. In their system, the phosphodiester DNA backbone of an anchoring strand of DNA is enzymatically cleaved. The cleavage results in a single strand of DNA preferentially hybridizing the next intact anchor strand, without the option of going back a step.

Taking a protein-based approach to engineering, the 'lawnmower' [13] is a design that exploits a burnt-bridges mechanism, thereby functioning autonomously. Recently constructed, the lawnmower is expected to bias its own motion by enzymatically cleaving peptide substrates along a track. As shown in Fig. 3, the lawnmower consists of a quantum dot hub conjugated to multiple protease 'blades' via flexible linker molecules. Inspired by the concept of a self-steering lawnmower, this motor was designed to undergo biased motion towards uncleaved substrate. The proteases serve not only as blades but also as 'feet' that first bind, and then cut, their foot-holds (peptides) as they move, thereby effectively biasing motion of the entire complex. The lawnmower is expected to remain on the track as long as the individual foot-binding events occur before complete detachment. The construct is entirely modular. The attached proteases can be switched, the hub size can be increased, and the linker arms connecting the blades and hub can be lengthened or shortened. On a large 2D surface, the motion is expected to resemble a self-avoiding walk, while constraining the track to 1D should result in directed motion. The modular design allows researchers to explore the influence of individual components on motor performance.

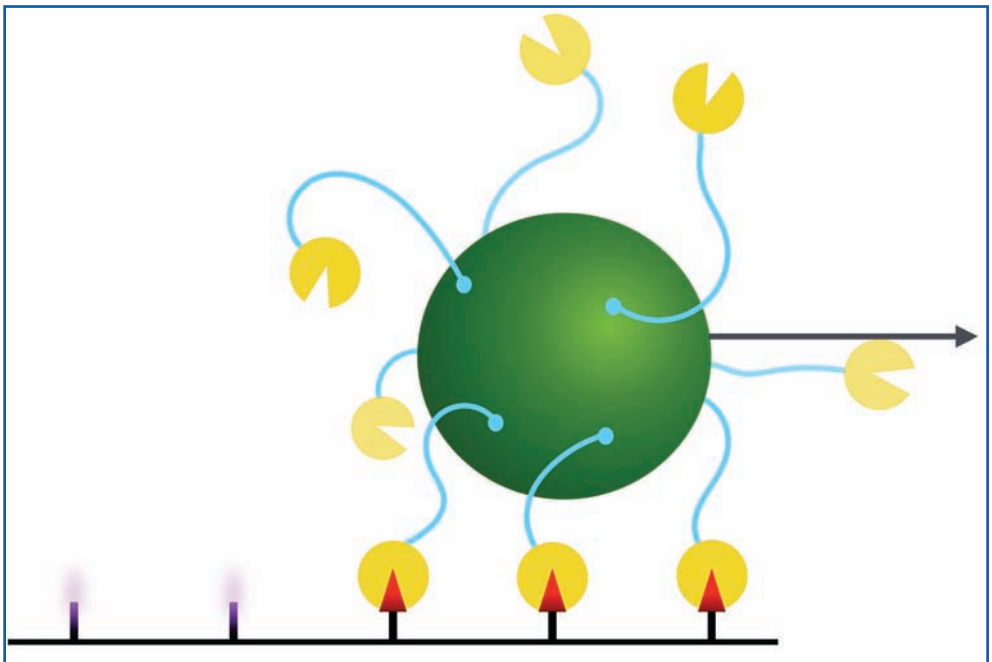

Fig. 3 The Lawnmower. Multiple proteases are coupled to a central quantum dot hub (8 nm in radius) that can be observed via fluorescence. The substrate track is comprised of peptides that contain a recognition sequence for the protease. Following binding, the protease cuts this sequence in two, with one portion diffusing into solution. Proteases then bind to uncleaved peptides; this biases the motion of the lawnmower in a burnt-bridges fashion. Detection of motion and of track cleavage can be performed using fluorescence: the portion of the peptide released by cleavage contains a quencher, thus resulting in fluorescence emission from the fluorophore remaining on the track-associated peptide stub. Because of the different spectral properties of the quantum dot and track, the motion of the motor can be tracked and correlated with cleavage of substrate, which provides a read-out of mechanochemical coupling.

**TABLE 1**
TERMINOLOGY

| | |
|---|---|
| **Ligand** | A small molecule that can bind to a protein and alter its function |
| **ATP** | Adenosine triphosphate; a source of chemical free energy used to fuel various cellular processes |
| **ADP** | Adenosine diphosphate; a product of ATP hydrolysis |
| **Peptide** | A molecule comprised of covalently linked amino acids |
| **Protease** | A protein that hydrolyzes the covalent bonds that link amino acids together |
| **Hydrolysis** | The water-facilitated cleavage of chemical bonds |

## CONCLUSIONS

There are many examples of artificial motors being pursued by researchers, each ingeniously designed to walk directionally along a track. In this article we covered examples of biological motors, motile DNA machines, and designs of protein-based synthetic molecular motors. These molecular devices, each in its own way, allow us to learn about the complex biological systems that led to their inspiration. Engineering artificial motors will not only help us understand biological processes, but also holds promise to create new technologies on the nano-scale. For example, DNA devices may have use as sensors for medical applications [14]. Although the field of synthetic nano-scale machinery is still in its infancy, it has great promise for future applications. We are only now realizing the great potential of these devices. There is still a lot to learn.

## ACKNOWLEDGEMENTS

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# RESOLVING BIOLOGY BEYOND THE DIFFRACTION LIMIT WITH SINGLE-MOLECULE LOCALIZATION MICROSCOPY

BY NAFISEH RAFIEI, DANIEL NINO, AND JOSHUA N. MILSTEIN

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Optical imaging provides a window into the microscopic world, but the level of observable detail is ultimately limited by the wavelength of light being employed. This "resolution limit" or "diffraction limit" results because light diffracts as it passes through an aperture, such as the objective of a microscope. The minimum separation distance at which two point sources of light are distinguishable can be quantified by the Abbe resolution limit:

$$\text{Abbe Resolution}_{x,y} = \frac{\lambda}{2\text{N.A.}}, \qquad (1)$$

where $\lambda$ is the wavelength of light and N.A. is the numerical aperture of the imaging lens [1]. In practical terms, using visible light and a high-N.A. objective, we are limited to resolving structural detail on the order of hundreds of nanometers. This was thought to be a fundamental limit of light microscopy, but in the past decade a number of ways to move beyond the diffraction limit have been devised. It's not that physicists[1] figured out how to break the diffraction barrier, rather they did what clever scientists do when faced with an insurmountable obstacle. They found a way to get around it.

## SINGLE-MOLECULE IMAGING

A number of techniques have recently been developed to circumvent the diffraction limit. They come bearing a variety of acronyms such as structured illumination microscopy (SIM) [2,3], stimulated emission depletion (STED) microscopy [4], photo-activated localization microscopy (PALM) [5], direct stochastic optical reconstruction microscopy (dSTORM) [6], and so on. Here we will focus on techniques like the last two in this list, PALM and dSTORM, which rely upon imaging photoswitchable fluorescent molecules. We refer to these techniques (there are a dozen or so more) as single-molecule localization microscopy so that the reader has to only remember one acronym (SMLM).

**SUMMARY**

**By harnessing the physics of photoswitchable dyes and fluorescent proteins, localization microscopy provides a window into the nano-world of biology.**

SMLM can produce images of structural detail an order-of-magnitude finer than diffraction limited microscopy. The technique relies upon precisely locating the position of single, fluorescent labels (Fig. 1). If the system being imaged only contained a single label, say an organic dye, the intensity distribution (or point-spread function (PSF)) of the dye would essentially be an Airy pattern. The central maximum of an Airy pattern is well approximated by a Gaussian, which may be fit to the PSF to obtain the spatial coordinates of the dye. In fact, the precision of this measurement, or the "localization precision", is primarily limited by the number of photons emitted from the dye, and scales like $s_{x,y}/\sqrt{N}$, where $s_{x,y}$ is the diffraction limited half-width of the PSF (i.e., the standard deviation of the fitted Gaussian) and $N$ is the number of collected photons. A more accurate quantification of the localization precision is given by the following formula [7]:

$$\sigma^2_{x,y} = \frac{s^2_{x,y} + \frac{a^2}{12}}{N}\left(\frac{16}{9} + \frac{8\pi(s^2_{x,y} + \frac{a^2}{12})b^2}{Na^2}\right), \qquad (2)$$

which depends on the pixel size $a$ (nm), level of background noise $b$ (photons/pixel), standard deviation of the PSF $s_{x,y}$ (nm), and number of collected photons $N$.

If there are multiple dyes within close proximity to one another, however, their PSFs will overlap and it will no longer be possible to simply fit the intensity distribution to localize the dyes. The trick, and it really is a trick, is to use photoswitchable dyes so that only a sparse subset of the dyes ever fluoresce at one time (Fig. 2). If, on average, only a single fluorophore emits photons at any one time in a diffraction-limited area, then each dye may be localized by fitting the PSF as before. In this context, we often speak of the duty cycle of the dye

$$DC = \frac{T_{ON}}{T_{OFF}}, \qquad (3)$$

1. The 2014 Nobel Prize in Chemistry went to Eric Betzig, Stefan W. Hell and William E. Moerner for developing super resolved microscopy. All three have physics degrees.

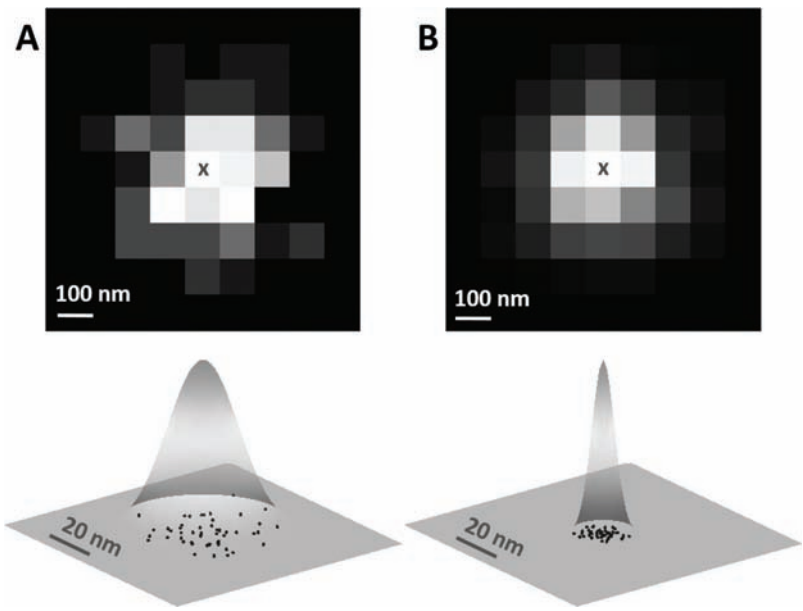


Fig. 1 (Top) Pixelated, camera images of a single fluorophore that emits A) $N = 100$ and B) $N = 1000$ photons. The cross (x) represents the fitted center of the intensity distribution (i.e., a localization). (Bottom) Multiple localizations of the fluorophore are represented by the blue circles and are Gaussian distributed. The width of the distribution (or localization precision) scales like $\sim 1/\sqrt{N}$. The localization precision is significantly reduced for the brighter fluorophore (A) compared to (B).

which is simply the ratio of the time the dye spends emitting photons ($T_{ON}$) to the time it remains dark ($T_{OFF}$). On average, so that the dyes' PSFs don't overlap, the duty cycle should scale like $1/M$ where $M$ is the number of dyes within a diffraction limited area. Many dyes can be tuned to exhibit duty cycles of $10^{-4}$-$10^{-5}$, which is required for high-resolution imaging [8].

With the current state-of-the-art in SMLM, single dye molecules can be localized with a precision of a few tens of nanometers in the lateral direction. In addition, there are a number of ways to extend SMLM to improve the depth resolution, and thereby perform full 3D imaging [9-11], although the localization precision is slightly worse than the lateral case by a factor of 2 or 3 times, dependent on the approach.

## MAKING A FLUOROPHORE BLINK PROPER

As mentioned, the key to SMLM is the ability to actively control the fluorescence emission of photoswitchable fluorophores so that the emission is sufficiently sparse. This can be achieved in a number of ways; for instance, by causing the dyes to intermittently blink through reversibly occupying a long lived dark state, cycling the fluorescence of a portion of the labels between two different wavelengths, or photoactivating a subset then rapidly photobleaching the emitters.

Let's consider PALM imaging [5], which makes use of inherently photoswitchable fluorescent proteins. A common fluorophore used in PALM is mEOS, which is a green fluorescent protein, but when exposed to near UV light (e.g., 405 nm) a fraction of the fluorophores will behave like a much redder dye and can be excited with a 561 nm laser line. A PALM experiment consists of activating a random subset of the fluorophores into the red channel, imaging those fluorescent proteins, then quickly photobleaching them. A new subset of fluorophores is activated, imaged, bleached, and the cycle repeats. PALM can be performed in fixed or live cells, albeit the requirement of cycling through repeated rounds of localization limits its utility in actively growing, functioning cells. Still, PALM is often used as a way to track proteins within live cells or to obtain rough images of structures that show slow dynamics.

Another approach is to "inactivate" all but a small subset of fluorophores while imaging. Fortunately, most all fluorophores display fluorescence intermittency (i.e., blink) by occasionally transitioning to a triplet or dark state via intersystem crossings before transitioning back to the singlet ground state, often through a non-radiative decay [12]. The time scale of these blinking events, however, is usually on the order of milliseconds or less. The idea of extending the time scale of the fluorescence intermittency is one of the key advances that paved the way for SMLM techniques. As an example, dSTORM, which employs organic dyes such as Alexa-647 or Cy5 [6], makes use of nonfluorescent, long-lived radical ion states beyond the usual triplet state. These dark states, which appear in many commercially available dyes when exposed to millimolar concentrations of thiolating compounds (e.g., beta-mercaptoethanol (BME) or cysteamine (MEA)), can display off times (i.e., when the dye does not fluoresce) of several seconds (Fig. 3).

## IMAGING THE BACTERIAL PROTEOME

SMLM microscopy is able to provide unprecedented structural detail with visible light microscopy. While the technique has found a range of applications, bacteria are a particularly suitable target because structure within a bacteria was previously inaccessible to light microscopy due to the micron size of these cells. Our lab uses SMLM to try to understand how the organization and packaging of the nucleoid (i.e., bacterial chromosome) affects cell function. In particular, we have focused on the

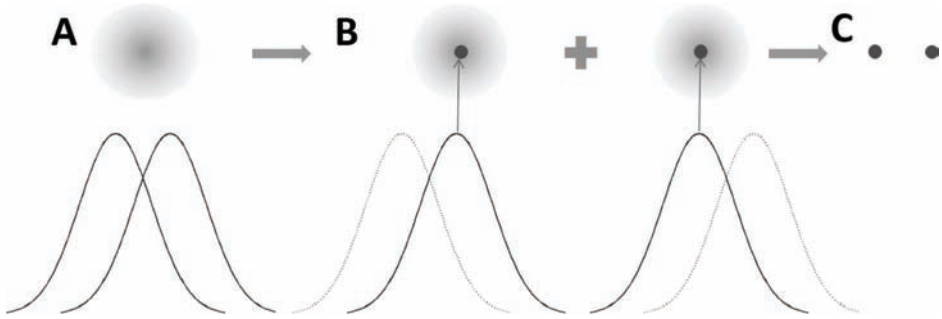


Fig. 2 The solid and dotted lines indicate the fluorescent (ON) and non-fluorescent (OFF) states, respectively. A) If the distance between two fluorophores is less than the Abbe limit, they are not resolvable. B) When only one of the fluorophores is in the ON state a 2D Gaussian can be fitted to the PSF to localize the corresponding fluorophore (red circle). C) The process is repeated for the other fluorophore and the positions are assembled to generate a high-resolution image.

arrangement of highly abundant nucleoid associated proteins (like H-NS, HU, and StpA), which package the bacterial chromosome, similar to histones in eukaryotes, while coordinating the expression of a multiplicity of genes [13].

These proteins may serve as environmental sensors that reorganize the chromosome under different environmental conditions, activating networks of genes to assist the cell in adapting to and colonizing its environs. SMLM provides a window into the global organization of these proteins, as well as allowing us to explore stochastic effects such as cell-to-cell variability within a population of bacteria (Fig. 4). The technique can also be combined with methods for visualizing chromosomal loci, such as DNA fluorescence in-situ hybridization (FISH) [14], which allows us to correlate the arrangement of these nucleoid binding proteins with the position of genes within the chromosome.

## QUANTITATIVE MICROSCOPY AND MOLECULAR COUNTING

Beyond imaging, a promising application of SMLM is as a method to quantify protein or nucleic acid abundance at a single cell level. Molecular counting with SMLM would be particularly powerful in single-cell genomic and proteomic applications. For instance, SMLM should be able to quantify low numbers of amplicons, which would enable a reduction or even elimination of the amplification stage required by current techniques for measuring single-cell DNA or RNA abundance, greatly increasing both the accuracy and reliability of these techniques. Several groups are now working on extracting accurate molecular counts from SMLM data in the hopes that the technique becomes the future gold standard for counting molecules [15-17]. Unfortunately, a number of complications still need to be overcome before SMLM is a viable approach to molecular counting. For instance, the photophysics of a fluorophore may be altered by environmental conditions (such as pH) within a cell or by fixation protocols. Moreover, many commonly used SMLM fluorophores are only around 50-60% active [16], which means that a large portion of the sample will go undetected. This, however, should improve as improved photoswitchable fluorophores are developed. And then there's the issue of generating an accurate table of single-molecule localizations. In practice, especially in samples where there are many aggregates or clusters of proteins, the PSF of the labels will begin to overlap degrading the reliability of the SMLM data set. In some cases, the duty cycle may be further lowered to reduce this overlap, but at the cost of an even even longer acquisition time. Improved localization algorithms [7,18] as well as increasingly complex models [19,20] of the fluorophore photophysics may be a better approach toward alleviating this issue.

## CONCLUSION

SMLM has pushed visible light microscopy far beyond the diffraction limit, shedding light on an ever increasing number

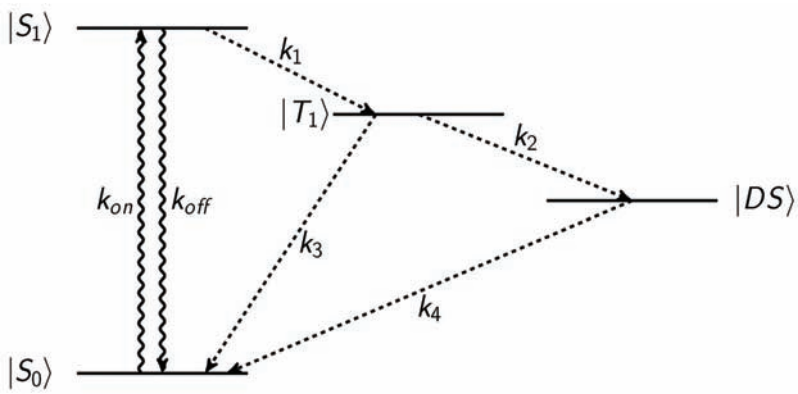


Fig. 3 Fluorescence occurs for transitions between the ground $|S_0\rangle$ and excited $|S_1\rangle$ singlet states. Intersystem crossings can lead to a spin flip transition into the $|T_1\rangle$ triplet state, but these states are usually short lived. Long lived, nonradiative, dark states $|DS\rangle$, however, can be induced in many fluorescent molecules.

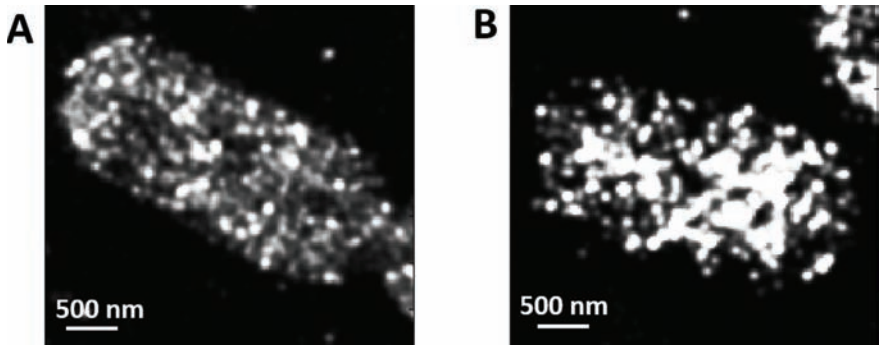


Fig. 4 A) PALM image reconstruction of fluorescently labeled H-NS in fixed E. coli bacteria. B) Following cold shock, H-NS is seen to reorganize along the bacterial chromosome into a much more clustered pattern of binding. The localization precision in these images is ~ 30 nm.

of biological questions. New and improved fluorophores are continually being developed that are more stable and yield more photons, ever increasing the resolution and speed of this technique. Algorithms for improving the reliability of localization tables (i.e., the acquired list of single-molecule localizations), especially within dense, inhomogeneous samples, are continually being developed as are image analysis tools for extracting quantitative knowledge from SMLM images. Moreover, SMLM can be combined with light-sheet or two-photon imaging to provide super-resolved images deep within samples such as biofilms or the cell nucleus. Finally, quantitative approaches such as molecular counting are gaining traction as an alternative modality for this single-molecule technique, making it increasingly relevant for the rapidly expanding fields of single-cell genomics or proteomics.

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# WATCHING SINGLE BIOLOGICAL MOLECULES FOLD USING LASER TWEEZERS

BY MICHAEL T. WOODSIDE

**SUMMARY**

**Using optical tweezers to unravel structures in biological molecules, we can watch single molecules fold directly, providing new physical insight into this crucial biological process.**

## THE FOLDING PROBLEM AND ENERGY LANDSCAPES

One of the remarkable features of biological molecules like proteins, DNA, and RNA is that they can form intricate three-dimensional structures. These molecules are alike in being linear polymers, consisting of simple chains of amino acids (proteins) or nucleotides (DNA and RNA). After synthesis in the cell, however, these chains "fold" into a vast diversity of shapes, from the elegant simplicity of the DNA double helix to the magnificent complexity of the ribosome, the enzyme that makes all proteins. Such structural diversity is the essential feature that underlies the functional versatility of these molecules, because of the tight link between structure and function. Understanding how biomolecules fold is critical: each molecule must fold into the correct structure to ensure correct function, and "misfolding" (forming the wrong structure) is linked to a wide variety of diseases [1].

The main challenge in solving the folding problem is not our understanding of the interactions within the polymer chain and between the polymer chain and its aqueous medium that drive folding, but rather their combinatorial complexity. A classic thought-experiment from Cyrus Levinthal showed that even for a small protein, a random search for the correct structure would take trillions of universe lifetimes, in contrast to the typical folding timescale of milliseconds to minutes [2]. The resolution to "Levinthal's paradox" is that folding does not involve a random search—instead, the search is biased by the favorable energies of native (correct) interactions within the chain as they form during folding. The modern picture of folding, arising from the theory of glasses, views folding as a search over a 'landscape' representing the energy of the molecule as a function of all possible configurations, where the search is driven by thermal diffusion within the conformational space [3]. This landscape is typically funnel-shaped (Fig. 1A), guiding the search rapidly and reliably to the native structure. Understanding how structure forms thus reduces to understanding the shape of the energy landscape and the paths taken across it during folding.

A key feature of folding landscapes is that there are usually energy barriers that must be crossed before reaching the native state. Such barriers arise from the delicate balance between enthalpy and entropy embodied in folding: whereas the interaction enthalpies favour the folded state, the entropy of the polymer chain favours the unfolded state. Stabilisation from enthalpy gains is offset to a large extent by destabilisation from entropy losses during folding, but these changes are not synchronised, producing barriers in the free energy along the way to the native state (Fig. 1A). These barriers, representing unstable conformations known as transition states, dominate the dynamics of the folding by acting as kinetic bottlenecks in the folding reaction, and they define the folding mechanism via the set of conformations visited during the structural transition.

Energy barriers create a critical challenge for experimental studies of folding, however: being unstable, they are hardly ever occupied. Molecules spend almost all of their time folded or unfolded (or possibly in metastable intermediates), and it has proven extremely difficult to observe them in the middle of the folding transition. Unfortunately, this is the most interesting part of the reaction, since it tells us how the self-assembly happens! Experimentalists have thus been limited to deducing the properties of the transition states indirectly, by observing how changes of experimental conditions (e.g., temperature, pressure, chemical denaturants, mutations, . . .) alter the lifetimes of the visible states and then modeling the effects on the transition states [4].

## SINGLE-MOLECULE APPROACHES TO TRANSITION PATHS

Advances in experimental methods in the last few years, in particular methods for observing the folding of single

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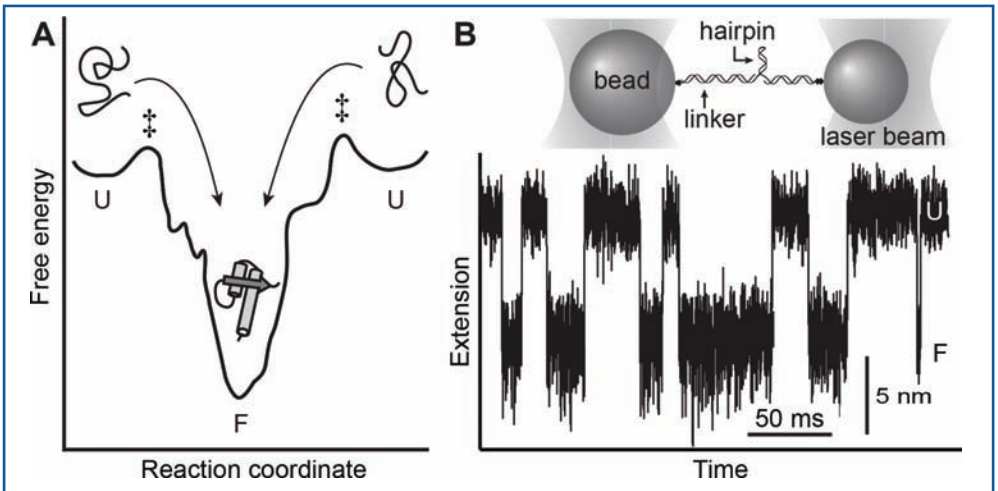


Fig. 1 (A) Cartoon of energy landscape for folding, showing change in free energy from unfolded (U) to folded (F). Landscape is funnel-shaped, but contains barriers (‡) dominating folding kinetics. (B) Folding trajectory of DNA hairpin measured with optical tweezers (adapted from Ref. [11]). Hairpin hops in equilibrium between F (low extension) and U (high extension). Inset: schematic of measurement configuration showing hairpin connected via linkers to beads held in traps.

molecules, have made it possible to characterise the paths followed during folding transitions more directly. Single-molecule approaches are necessary in order to avoid the effects of averaging, because folding transitions cannot be synchronised in an ensemble of molecules. To observe transition paths successfully, however, additional technical hurdles must be overcome: obtaining high enough precision to distinguish different structural states along the paths, high enough time resolution to capture the molecule during the very fleeting transition paths, and a wide enough dynamic range to cover the typical thousand- to million-fold disparity between the transition time and the lifetime of the folded and unfolded states.

Pioneering work using energy transfer between fluorescent dyes attached to different parts of a protein (thereby allowing structural changes during folding to be monitored) showed that the average time for crossing the transition paths could be measured by carefully analysing the statistics of the photons emitted by the dyes [5,6]. These measurements found an average transition time on the order of 2–10 μs, but they could not distinguish individual transition paths, owing to limitations in the dye photophysics. We took a different experimental approach, using laser tweezers to induce and monitor structural changes via single-molecule force spectroscopy (SMFS). Laser tweezers make use of the gradient force induced in small dielectric objects by the electric field gradient in a tightly-focused laser beam to trap micron-sized beads near the focal point—effectively, the tweezers act as a Hookean spring in three dimensions made of light [7]. In SMFS measurements, each end of a molecule is attached via linkers to the beads held by the tweezers (Fig. 1B inset), allowing force to be applied across the molecule. When the molecule unfolds, the unstructured part is stretched out under the applied tension, resulting in a change in the extension of the molecule [8] (Fig. 1B). Laser tweezers provide an excellent probe for transition paths: extensions can be measured with A-scale resolution, time resolution of $<10$ μs can be achieved by using small beads and stiff traps, and hundreds or thousands of transitions can be observed in a single molecule by applying just enough tension so that the molecule is poised in equilibrium between the folded and unfolded states, hopping back and forth repeatedly. Furthermore, SMFS provides one of the most reliable ways to measure energy landscapes directly, rather than deducing them from modeling [9].

## TESTING FOLDING THEORIES WITH TRANSITION-PATH MEASUREMENTS

We recently applied this approach to make the very first measurements of transition paths in proteins and nucleic acids [10,11]. Making use of improvements in instrumental time resolution, we extracted the paths followed through the transition states directly from trajectories of the extension (as in Fig. 1B) by picking out the parts of the trajectory where the molecule moved all the way from unfolded to folded or vice versa. Examples of the transitions observed in a DNA hairpin, a model system for studying folding that consists of a simple stem-loop (Fig. 2A inset), reveal extremely rich and varied behaviour (Fig. 2A): the timescale for individual transition paths can vary by over an order of magnitude, the local velocity along the paths varies greatly, and brief pauses of variable duration can occur at varied locations. The hairpin can even be seen shuttling back and forth across the energy barrier, providing direct evidence of the diffusive nature of the structural search and definitively confirming that folding cannot be described by classic transition-state theory (as once thought), which does not allow for barrier re-crossing. The 1000-fold disparity between the duration of the transition paths and the lifetime of the unfolded and folded states can be seen clearly by comparing Figs. 1B and 2A. To verify that the signals in the transition paths arise from hairpin folding and not instrumental noise, we repeated the measurements with beads connected by handles but no hairpin, finding that the transitions were much faster and less variable (Fig. 2B).

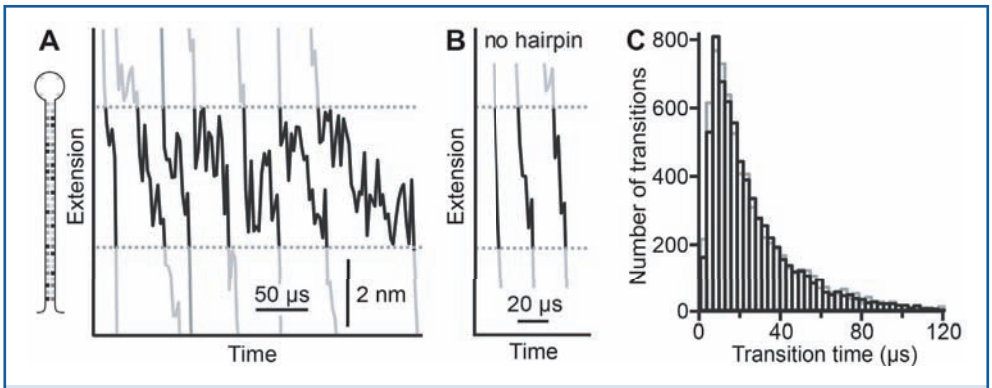


Fig. 2 (A) Representative folding transitions for DNA hairpin showing diverse behaviour. Dotted lines denote boundaries of barrier region. Inset: hairpin structure (dark grey: G:C base-pairs, light grey: A:T base-pairs). (B) 'Transitions' obtained from reference construct without hairpin, illustrating the instrument time response and noise levels. (C) Distribution of transition path times for folding (black) and unfolding (grey). Adapted from Ref. [11].

Measurements like these open an exciting new window onto the biophysics of folding: by allowing the most important parts of folding transitions to be observed directly, they permit the basic physical processes involved to be probed and hence enable experimental testing and refinement of the theory of folding. Furthermore, they hold the promise of providing critical new insights into the mechanisms of folding, by identifying the specific sequence(s) of transition states that determine how a given molecule folds. As an example, we used measurements of the time required to cross the transition paths (Fig. 2C) to test some fundamental features of folding theory. Looking at thousands of transition paths, we found that the average transition time measured directly [11] agreed well with the result predicted for diffusive motions across the energy landscape profile reconstructed experimentally from the equilibrium thermodynamics of the folding [12,13]. Moreover, the coefficient of diffusion for motions along the energy landscape, as extracted from the exponential decay of the transition-time distribution (Fig. 2C), matched the value expected from classic theories of chemical kinetics based on the observed lifetimes of the folded/unfolded states, showing that the theory is consistent with experiment across a wide range of timescales.

We also tested the basic physical picture of folding as a diffusive search over an energy landscape by probing the statistics of the transition-path occupancy—the fraction of time spent at each location within the transition states. Remarkably, although this picture has been tested quantitatively by both computational simulations and experiments combined with modeling, there were no purely experimental tests proving that the observed molecular motions agreed with the expectations for ideal diffusion over the landscape measured for that same molecule. We performed such tests for both model DNA hairpins [14] and the protein that causes "mad-cow" disease, PrP [15], using the transition-path occupancy to determine the conditional probability of being on a transition path at any given value of the molecular extension, $p(\mathrm{TP}|x)$. Theorists predict that for ideal diffusion, $p(\mathrm{TP}|x)$ should be related to the landscape via the splitting probability, $p_{\mathrm{fold}}(x)$, the likelihood that the molecule reaches the folded state before the unfolded state starting from extension $x$ [16]. Intuitively, at the barrier top $p_{\mathrm{fold}} = ½$, whereas $p_{\mathrm{fold}} \sim 1$ near the folded state and $\sim 0$ near the unfolded state (Fig. 3, inset). Calculating $p(\mathrm{TP}|x)$ from the statistics of the transition paths for these molecules (Fig. 3, black) and comparing to the theoretical expectation for ideal diffusion (Fig. 3, grey) over the measured landscapes (Fig. 3, dashed line) [12,17], we found that indeed there was quantitative agreement for these molecules [14,15].

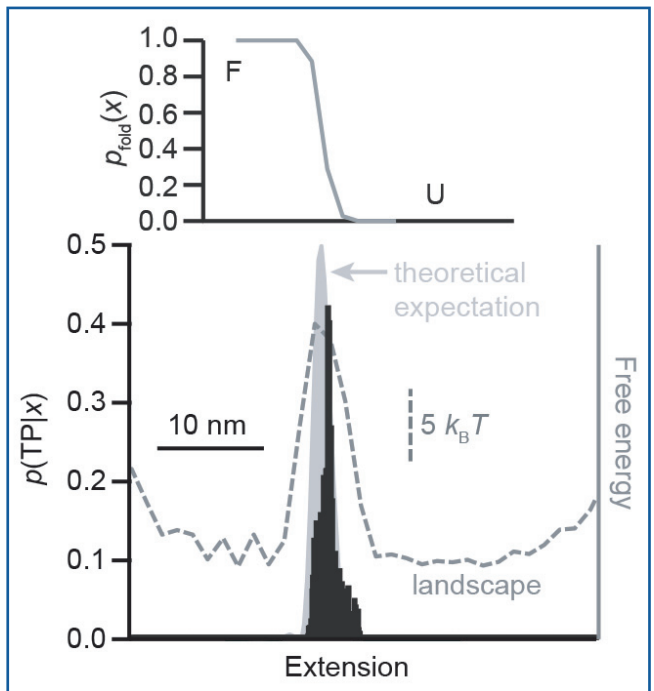


Fig. 3 Conditional transition-path probability (black) for folding of PrP matches theoretical expectation (light grey) calculated from pfold (inset) based on experimentally measured landscape (dashed line), validating basic physical picture of protein folding. F: folded state, U: unfolded state. Adapted from Ref. [15].

## FUTURE OUTLOOK

Of course these examples only give a small taste of what can be done with transition-path measurements, and many exciting facets of transition paths remain to be explored. The rich phenomenology of the pauses in the transition paths, which briefly capture the molecule in the transition states, will doubtless reveal fascinating insights into the transition-state properties, and will allow the models heretofore used to characterise transitions states indirectly to be tested directly. Direct observation of transition paths also opens up the ability to see if there are multiple types of pathways and barriers involved in the folding (even if only one dominates) and if so to characterise them individually. Furthermore, opportunities abound for integrating transition-path measurements with long-duration atomistic simulations [18], since the timescales for both are converging. Such integration can amplify the insights that can be obtained from either method alone, both validating the simulations and helping to overcome intrinsic limitations on the structural information available from the experiments. Finally, future work should clarify the effects of the applied force on the transitions, from examining potential kinetic artifacts [19] to testing whether the tension biases the choice of paths. The next few years should thus see many exciting advances in our understanding of folding as transition-path measurements become more widely used.

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# PHYTOGLYCOGEN NANOPARTICLES: EXCITING SCIENCE AND PROMISING TECHNOLOGIES FROM NATURE

BY JOHN R. DUTCHER, MICHAEL GROSSUTTI, JOHN ATKINSON, BENJAMIN BAYLIS, HURMIZ SHAMANA, ERIC BERGMANN, JONATHAN NICKELS, AND JOHN KATSARAS

**SUMMARY**

**Nature offers amazing examples of nanostructured molecules and materials. We describe the unique structure, hydration and applications of naturally occurring phytoglycogen nanoparticles.**

Nanoscale science and technology is a very active and multidisciplinary area of research. A significant amount of this research is inspired by materials and mechanisms found in nature [1]. Some of the most familiar examples from nature that rely on nanoscale structure include the iridescence of butterfly wings, the self-cleaning nature of lotus leaf surfaces, and the adhesive nature of gecko feet. At the level of single molecules, biology offers lipids, proteins and DNA, which demonstrate an exceedingly high level of precision of expression and structure that is required for their proper biological function. Assemblies of these molecules result in elegant nanoscale structures such as lipid vesicles, protein filaments, and molecular motors that form the basis of many essential biological processes, and are exquisite examples of self-assembled nanotechnology found in nature. It is certainly true that much can be learned from nature to produce new nanostructured materials for new technologies. As physicists, it is fascinating to look in detail at the amazing structures and mechanisms that have evolved at the molecular level.

In the present article, we focus on a special biopolymer, phytoglycogen. This is a highly branched, water-soluble polymer of glucose produced by plants such as sweet corn and rice, and is analogous to glycogen, an energy storage molecule in animals. The molecules are chemically simple, but have a special dendrimeric or tree-like structure (Fig. 1) that results in compact, monodisperse nanoparticles with many applications in personal care, food and nutrition, and biomedicine.

The particles were originally discovered in the Dutcher Lab in a fundamental, multidisciplinary research project focused on bacterial adhesion. They were the waste product of a multistep chemical procedure and it was their special optical scattering (opalescence) that led to their serendipitous discovery. Our realization that similar particles, phytoglycogen, are produced by sweet corn led to the promising sustainable nanotechnology described in the present article.

The applications of phytoglycogen rely on exceptional properties that emerge from the structure of the phytoglycogen nanoparticles and their interaction with water, such as a remarkable capacity to retain water, and low viscosity and exceptional stability in aqueous dispersions [2,3]. To investigate the origin of these properties, we have chosen physical characterization techniques that are particularly well suited to the study of polysaccharides and water, such as neutron scattering, infrared spectroscopy, rheology, atomic force microscopy and ellipsometry. Below, we describe how these measurements have revealed important information about the structure and hydration of phytoglycogen nanoparticles. The results of these experiments provide new insights into the fundamental physical properties and promising applications of this remarkable sustainable nanomaterial.

## STRUCTURE AND HYDRATION OF NANOPARTICLES

Neutron scattering is ideally suited to the study of biological materials at the molecular level [4]. Neutrons have a small wavelength (of the order of an Angstrom), and therefore scattering experiments provide information down to the molecular, and sometimes, the atomic level. Unlike x-rays, neutrons scatter well from low atomic number elements and so are well suited for the study of biological materials. In addition, deuteration (substituting deuterium D for hydrogen H) can be used to enhance or suppress scattering, since neutrons scatter with the opposite phase from H and D atoms. For colloidal dispersions of particles in water, deuterated water is used to vary the scattering contrast between the particles and the solvent in an experiment known as a contrast series, and to avoid the increase in the background signal due to the large incoherent scattering cross-section of hydrogen. In small angle neutron scattering (SANS), the scattering intensity $I$ is measured as a function of

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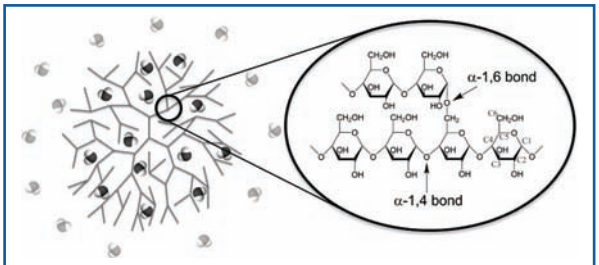


Fig. 1 Schematic cross-section of highly branched phytoglycogen nanoparticle (left), distinguishing between water molecules inside (hydration water) and outside the nanoparticle (bulk water, shown in muted greyscale). The chemical structure of a small portion of the nanoparticles is shown on the right. Adapted from Ref. [2].

scattering wavevector $q$ (such as the curves shown in Fig. 2A). SANS can be used to determine the particle diameter for dilute dispersions, and the average spacing between particles for concentrated dispersions [2]. These measurements revealed a particle diameter of 35 nm for the phytoglycogen nanoparticles, with a small polydispersity of ~10 %. This value of the particle diameter is consistent with direct imaging of the nanoparticles using atomic force microscopy (AFM) [5]. In measurements on high concentration dispersions, we observed that the particle spacing was equal to the particle diameter at a concentration of ~20 % w/w, indicating the onset of jamming of the nanoparticles that was consistent with the results of rheology measurements on aqueous dispersions [6].

Contrast series were performed in which the ratio of $D_2O$:$H_2O$ in the solvent was varied between 0 and 100% (Fig. 2). We found that, at a particular value of % $D_2O$, the neutron scattering length density (NSLD) of the solvent was almost exactly matched to the NSLD of the particles, suppressing the scattering intensity. Specifically, we observed a quadratic dependence of the total scattering (Porod) invariant $Q^* = \int q^2 I(q) dq$ on % $D_2O$ with a single minimum in the scattered intensity. The results for 12.9 % w/w phytoglycogen are shown in Fig. 2, and a similar dependence was observed for a higher phytoglycogen concentration of 22.4 % w/w [2]. These results indicated that the radial particle density of the nanoparticles is uniform, confirming that the flexible glucose chains uniformly fill in the space available within each nanoparticle [2].

The molecular weight and water content of the nanoparticles could also be determined from the SANS experiments. By dividing the sum of the atomic scattering lengths corresponding to the chemical formula for a glucose monomer by the neutron scattering length density measured at the contrast matching condition, we were able to calculate the glucose monomer volume. This value, together with the measured particle diameter, allowed us to determine the number of glucose units in each nanoparticle. By comparing the scattering at the contrast match condition with that in pure $D_2O$, we were able to determine that each nanoparticle sorbed ~250 % of its own weight in water [2].

Because of the high water content of the nanoparticles, the dynamics of hydration water inside the particles can be compared to that of bulk water outside the particles using

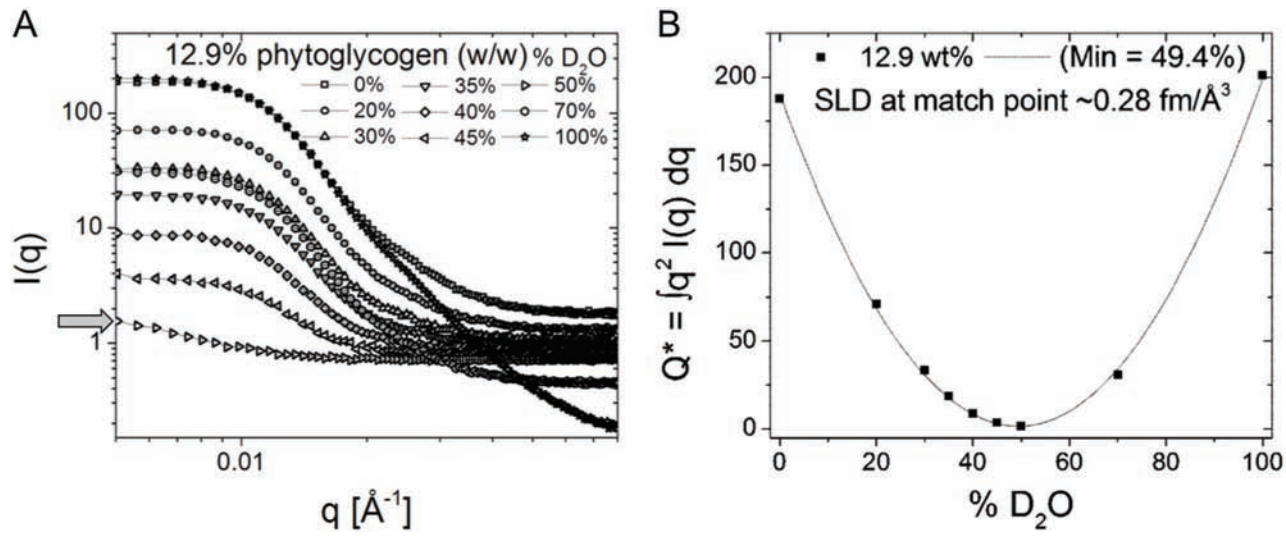


Fig. 2 SANS was used to determine the monomer volume and water content of phytoglycogen nanoparticles. (A) Scattering intensity $I$ versus scattering wavevector $q$ for 12.9% w/w phytoglycogen dispersions as a function of % $D_2O$ in the buffer. The horizontal arrow indicates the data measured for 50% $D_2O$. (B) The total scattering, as indicated by the total scattering invariant $Q^*$, shows a clear minimum near 50% $D_2O$. Adapted from Ref. [2].

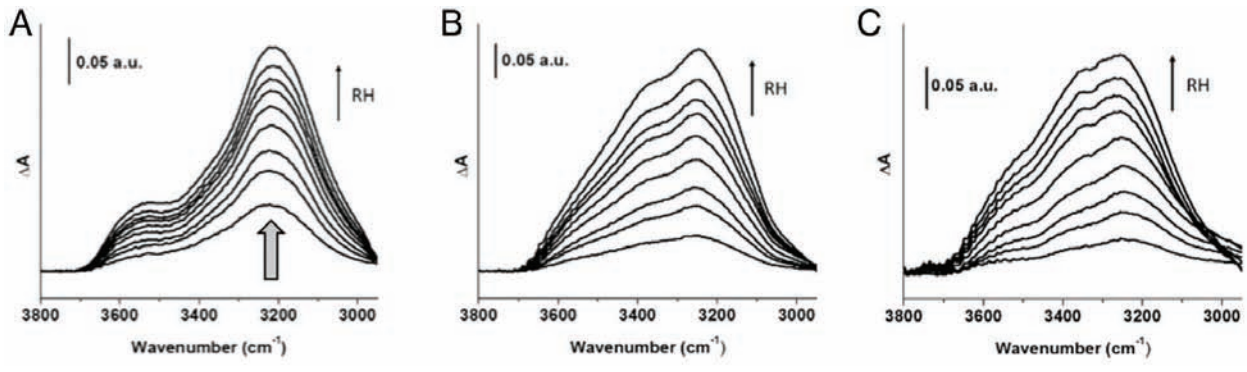


Fig. 3 Surface-sensitive infrared absorption difference spectra [$\Delta A = A(RH\%) - A(0\%)$] for (A) phytoglycogen, (B) hyaluronic acid, and (C) chitosan. The narrow arrows indicate the progression of spectra with increasing RH, and the wide arrow in (A) indicates the peak corresponding to highly ordered water. Adapted from Ref. [3].

quasi-elastic neutron scattering (QENS). QENS allows the observation of atomic/molecular motions on the time scale of tens of picoseconds to ~1 ns, over length scales of 0.3 to 3 nm, which is ideal for studying the diffusion of water. These measurements revealed a significant slowing down or retardation of the hydration water relative to bulk water by an average factor of $\xi \sim 5.8$. By performing the QENS measurements at different scattering wavevectors $q$, we observed that the retardation in the water dynamics was length-scale dependent [2]. This result showed for the first time that the measured value of the retardation factor $\xi$ depends on the length scale probed in the experiment, and may help to reconcile the often conflicting range of hydration water retardation factors reported in the literature for different experimental techniques [7]. QENS also provided an additional independent and consistent estimate of the amount of water in each phytoglycogen nanoparticle, showing that the particles sorbed 285% of their own mass in water. Taken together, the SANS and QENS measurements provided a wealth of essential information about the structure and hydration of the phytoglycogen nanoparticles.

To learn about the structure of the hydration water inside the phytoglycogen nanoparticles we used infrared (IR) spectroscopy, which reveals details of molecular vibrations. By focusing on the band corresponding to the OH stretching vibration, IR spectroscopy can be used to determine the relative amounts of different sub-populations of structured water. The OH stretching region in IR absorption spectra of hydrated polysaccharides is complicated because it contains contributions from different sub-populations of water as well as contributions from bonds within polysaccharides, e.g., C-H, O-H and N-H stretching. We eliminated the contribution from the polysaccharide bonds by subtracting the IR absorption spectrum collected for thin films of polysaccharides at a low value of relative humidity ($RH$) from spectra collected at higher $RH$ values [3]. This technique allowed us to compare the water structuring in chemically different polysaccharides. We found striking similarities between water structuring in two linear polysaccharides, hyaluronic acid and chitosan, and significant differences between the linear molecules and highly branched phytoglycogen (Fig. 3). In particular, the hydration water in the phytoglycogen nanoparticles is significantly more highly ordered and tightly bound than in the linear polysaccharides, as indicated by the large peak at ~ 3200 $cm^{-1}$ in the phytoglycogen spectra (Fig. 3). These measurements suggest that the high degree of branching in phytoglycogen leads to a much more well-ordered water structure, indicating the strong influence of chain architecture on the structuring of water.

We also studied the interaction of the nanoparticles with water by measuring the equilibrium swelling of ultrathin films of the nanoparticles [8]. Ellipsometry is a sensitive optical technique that is ideally suited to this measurement, allowing precise determinations of the thickness and index of refraction of thin films [9]. The swelling of polysaccharide films, produced by changing the RH of the air surrounding the films, provides insight into short-range repulsive forces acting between the chains at low RH, and hydration forces at high RH [10,11]. The hydration forces can be interpreted in terms of an exponential decay of the disjoining pressure acting across the film: $P = P_0 \exp(-D/\lambda_0)$. We find that the highly branched phytoglycogen nanoparticles have hydration properties that are intermediate to those of cellulose, a semi-crystalline glucose-based polysaccharide, and those of dextran, a less branched glucose-based polysaccharide. These results reiterate the strong influence of chain architecture on the hydration properties. We also compared the swelling results with IR measurements of the water structure, and we find a correlation between the structural rearrangement of the hydrogen-bonding network of the tightly bound hydration water and the inter-chain separation in the highly branched phytoglycogen nanoparticles [8].

## TECHNOLOGY TRANSFER AND COMMERCIALIZATION

The unique interaction of water with phytoglycogen nanoparticles suggests a wide range of promising technological applications. For example, the strong bonding of water to the nanoparticles provides a unique advantage for use as a moisturizing agent in cosmetics. In response to changes in *RH*, phytoglycogen nanoparticles will sorb and desorb water less readily than other polysaccharides. This provides an advantage for the use of phytoglycogen as a long-term moisturizing agent in cosmetics, with more consistent delivery of moisture in environments in which considerable changes in *RH* occur. Hydrophobic modifications of the nanoparticles extend the range of applications to solubilizing and stabilizing hydrophobic bioactive compounds in foods and medicines.

Initially, the technology was developed in the Dutcher Lab at the University of Guelph, supported by over $1M in government grants. The technology is now being commercialized by Mirexus Biotechnologies Inc. (MBI), which is a spinoff company from the Dutcher Lab [12]. Through its subsidiary companies Mirexus, Fortis Ingredients and Glysantis, MBI is pursuing opportunities in personal care, food and nutrition, and biomedical applications, respectively. MBI has been successful in raising $19M in investment, and operates an R&D facility in Guelph with 18 fulltime employees (8 PhDs). MBI works closely with a network of university researchers to gain new knowledge and to discover and evaluate new applications of the phytoglycogen nanoparticles.

## CONCLUSIONS

By applying sophisticated physical characterization techniques to the study of naturally occurring polysaccharide nanoparticles, we have learned in detail about the interplay between the unique particle architecture and hydration water. The results of these studies have also provided valuable input for the design of new technologies that are being commercialized by Mirexus Biotechnologies Inc. This work demonstrates the value of physics-based strategies for the discovery and characterization of sustainable nanomaterials.

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# CANADA: AN OUTSTANDING ENVIRONMENT FOR ACADEMIC TECHNOLOGY ENTREPRENEURS

BY ANDRE MARZIALI


## SUMMARY

**Opinions on the unique strengths and advantages of biotechnology entrepreneurship in Vancouver are presented, including government and academic support, availability of highly skilled persons, and general climate and culture.**


It seems to me that if Canadians have an illness it's the perpetual feeling that we are in second place in North America. While in terms of population, science funding and other variables this is clearly true, this doesn't have to be the case for, among other things, entrepreneurship.

While I've spent most of my life in Vancouver, Canada, I spent my graduate and post-doc years in Silicon Valley (1989-1998) – one of the most vibrant technology entrepreneurial hubs in the world. These days, I split my time between UBC and my biotech start-up, Boreal Genomics, a company that commercializes DNA enrichment solutions for early cancer diagnostics. I live in Vancouver but am frequently on business in the SF bay area, and have been immersed in its entrepreneurial culture now for several decades. Drop into any coffee shop in Palo Alto and you'll over hear a conversation among founders of some tech start-up that's just getting off the ground, or just been funded, or, for that matter, that is running out money. Regardless of the outcome, the fact is that in the Silicon Valley environment, starting a tech company is about as natural and routine as going for your first job interview. In the lab where I worked after my PhD (the Stanford DNA Sequencing and Technology Center) almost everyone working at the PhD or postdoc level was starting, or thinking of starting, a company to commercialize their work.

Upon returning to Vancouver in 1998, I instantly felt a massive difference in optimism about entrepreneurship. Starting a company here, I was told, is hard. There's no venture funding, it's hard to recruit management, etc. The reasons were many, and there was the occasional horror story attached to some of these statements. That's certainly not because Vancouver or UBC was new to tech entrepreneurship. The Physics department at UBC was already well known for some of the earliest spinoff companies from UBC, and Vancouver had numerous success stories. The problem, as far as I can tell, was, and maybe still is, simply one of critical mass and culture. I'm glad to see that it's finally changing, and thought it worthwhile fueling this change with some observations from my own experiences as to why Canada, Vancouver in my case, is a fantastic place for academics to start a technology company.

First of all, my experience with entrepreneurship at UBC highlighted an exemplary level of support for my endevaours from both the University and from my home department of Physics and Astronomy. It seems that Canadian academics at most institutions are allowed to work in their start-ups part time without endangering their academic positions. Unlike the US where some very strict limitations are put on this, in Canada, most universities accept the inevitable conflict of interest and are willing to put measures in place to manage it rather than outright disallowing it. This creates an environment where the technology inventors and pioneers can lead or play a strong role in the companies commercializing their own work, without taking a giant career risk. Any start-up has only small odds of being successful, and is sometimes fueled more by passion than anything else. Take away the one person that is most passionate about the technology and replace that person with a hired leader, and you've just decreased the odds of success. In Canada, we allow our technology inventors to play the critical role of champion in their ventures, which I suspect in many cases improves their odds.

Another excellent reason to start a technology venture in Canada is the impressive levels of government support. The National Research Council's Industrial Research Assistance Program (IRAP) provides excellent assistance for young companies that are bridging that gap between government sponsored academic research and venture capital. IRAP was absolutely critical during the early days of our start-up, helping us with anything from market research, to funding new hires and major projects. IRAP's support is relatively small compared to venture funding (typically up to a few $100,000s of funding) and typically requires matching expenditure by the company, but in the early stages of a company's life this is a critical boost. Boreal benefitted tremendously from the IRAP programs, which helped us grow the company to where we were ready to make a convincing pitch to investors. Mitacs and


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other organizations also offer student, or new hire, support programs that are worth taking advantage of.

Perhaps the most significant government support for our start-up though came from SR&ED (Scientific Research and Experimental Development Tax Incentive Program). Details of the program are available on their website, but in brief, SR&ED allows a fraction of research expenditures by Canadian controlled companies to be returned to the company as refundable tax credits. This is a fantastic program for early stage companies funded through substantial research contracts or venture funding, as it provides a significant amount of cash as long as enough money is flowing through the company. In our case, SR&ED support resulted in several million Canadian dollars flowing to Boreal in cash during the period our company was Canadian controlled, and additional amounts in non-refundable tax credits now that we are controlled by various non-Canadian investors. While these are impactful amounts of cash for a growing company, the SR&ED support seems to be a uniquely Canadian benefit (at least in North America), that can serve as a significant incentive for foreign investors.

This brings me to my next point: venture capital. It is certainly true that Vancouver has relatively little venture capital compared to other tech centers. Some other major Canadian cities are likely in better shape, but still presumably fall far short of what can be found on Sand Hill Rd. in Palo Alto. This shortage seems to be often cited as the fundamental reason why it is hard to start companies in Vancouver. I'm not sure how detrimental this really is though. When it came time for our young start-up to raise funds from venture investors, we started the search in the US, not Canada. While it is true that some US investors don't like to invest across the border, many do, making the geographical location of the capital relatively unimportant. We ended up raising substantial amounts of money from US and international investors, who also turned out to be outstanding mentors and directors of our company. Talking to other companies, this experience seems to be a bit of an outlier perhaps, but I don't think it's that hard to replicate if one focuses on a broad search for talented investors. We did also raise some money in Vancouver, which certainly helped us keep our Canadian controlled status as long as possible, but had the well been completely dry in Vancouver, we would have still been able to fill our fundraising rounds. The current reality is that a number of US investors are becoming aware of programs like SR&ED which in a sense provide instant return on their investment, and are therefore starting to take a closer look at investments in Canada. The bottom line is there is money available; it is largely a question of finding the right connections and having a compelling business opportunity.

Another significant incentive for US investors to fund Canadian companies is our efficiency with capital. Salaries in Canada are in Canadian dollars, and on a different scale from the over-inflated salaries of tech centers like Silicon Valley. There is also, in my opinion, a substantial difference in culture between start-ups in Canada (or perhaps anywhere) and those in Silicon Valley. Silicon Valley start-ups seem to love to spend money as a matter of culture. The approach seems to be to spend fast, grow fast, and hope for rapid success. This works very well sometimes, but can also lead to incredibly high rate of capital burn at inappropriate times. When our company had roughly equally sized groups in Vancouver and the SF bay area the burn rate of the bay area group was much higher than the Vancouver group due in part to salary discrepancies but also due to a difference in spending and hiring culture. When it came time to control our burn rate as we found we were too early in our target market, it was the bay area group our US investors chose to shut down, re-grouping the company in Vancouver where it continues to operate very effectively on a small fraction of our former US group burn rate.

Finally, any tech start-up lives or dies by the quality of the team it can assemble, and hiring talented people in the US tech centers is a major challenge. It's not that there's any lack of outstanding talent in the bay area for example; it's simply that competition from massive employers like Google and Facebook make it very hard for young start-ups to compete for talent. I have heard this pain expressed by sizable (and public) US biotech companies, complaining that R&D hiring in their area is extraordinarily difficult, to the point that they are considering establishing R&D sites elsewhere.

In Canada on the other hand, or at least in Vancouver in my experience, there is a broad talent pool of very young but very smart individuals with the kind of drive and enthusiasm that make start-ups successful. Most of our company was built on this talent – nearly all our employees came directly out of BC universities, or returned to BC after graduate work in foreign universities. The fact is Canada is a very nice place to live, and I believe we will increasingly see a trend of our young people retuning to live and work in Canada even after studying abroad. While salary differences remain to the US, many will see this offset by our fantastic environment, particularly for young people starting families.

In summary, I've tried to provide a bit of our story as encouragement to Canadian academics considering commercialization of their work. It is possible, it can be done at low personal risk, and it can be exceptionally rewarding. It's not for everyone, but Canadian scientists should know they're in an environment that is actually very well suited for company formation, and that can benefit from the best combination of Canadian culture and international funding. Our Engineering Physics program at UBC is now spinning off several companies a year founded largely by young graduates in their early twenties. The culture is definitely shifting toward entrepreneurship, and it's starting to feel realistic that in the not too distant future Canadian technology hubs like Vancouver could outcompete Silicon Valley.

# NANOPORES: ELECTRONIC TOOLS FOR SINGLE-MOLECULE BIOPHYSICS AND BIO-NANOTECHNOLOGIES

BY VINCENT TABARD-COSSA, KYLE BRIGGS, AUTUMN CARLSEN, MARTIN CHARRON, PHILIPP KARAU, ZACHARY ROELEN, AND MATTHEW WAUGH

Nanopores – nanometer-sized holes in ultrathin membranes – are driving a revolution in the life sciences and in medicine, where high-performance detection systems must achieve greater speed, sensitivity and accuracy. In this short review, we present the principles of solid-state nanopore-based single-molecule detection and discuss the challenges, which must be overcome in order for solid-state nanopores to transition from a leading candidate under development to a disruptive technology.

## INTRODUCTION

Dynamic phenomena in confined geometries, including the transport of charged biopolymers through nanoscale pores, are fundamental processes of life (e.g., the passage of mRNA through the nuclear pore complex; the secretion of proteins across cell membranes). Elucidating the fundamentals of biomolecular transport through nanopores is a fertile field of research for many physicists, and strategies for controlling molecular capture and passage will find numerous technological applications.

The last decade has seen significant advancements in nanofluidic devices to develop biomimetic systems and to study transport processes at the single-molecule level [1,2]. These studies have shed some light on the underlying mechanisms of polymer dynamics in pores [3]. In particular, exciting results have been obtained through the study of passage of nucleic acids through solid-state nanopores (ssNP) [4–7]. Nanometer-sized holes in thin dielectric membranes, ssNP have emerged as a versatile tool to investigate a wide range of phenomena involving DNA and proteins. They provide a confined space within which single molecules can be captured and electrically interrogated with high throughput. The basic concept behind single-molecule analysis using nanopores, illustrated in Fig. 1, is the following: when a small voltage bias (~100 mV) is applied across a nanopore in a membrane separating two chambers filled with a liquid electrolyte (typically 1M KCl), a charged molecule can be electrophoretically driven into the pore, resulting in a transient change in ionic current (~sub-nA) which provides information (e.g., length, size, charge, shape, and dipole) about the translocating molecule. In addition to single-molecule DNA sequencing [8], this concept exhibits great promise for other applications, including molecular counting [7,9]; scanning of local structures along DNA molecules [10] for specific protein detection [11], sequence-specific PNA binding detection [6], and investigation of single biomolecular interactions [12]. In the present article, we review the principles of nanopore-based single-molecule detection, the main hurdles in developing nanopore-based technologies for life sciences and medical applications, as well as the progress our laboratory has made toward providing innovative solutions to challenges in the nanopore field.

**SUMMARY**

**We review the principles and challenges of nanopore-based single-molecule detection for life sciences and medical applications, as well as the progress our laboratory has made toward providing innovative solutions.**

### Nanopore Signal, Passage Speed, and Capture

In a typical nanopore experiment, an applied voltage $V$ drives ions through a small pore of diameter $d_{pore}$ in a thin membrane of thickness $L$, separating two fluidic reservoirs filled with an electrolyte of conductivity σ. The passage of a biomolecule with diameter $d_{analyte}$ results in a current change expressed as $\Delta I = \frac{V\sigma\pi d_{analyte}^2}{4L}$. The nanopore size requirements strongly depend on the biosensing application of interest. The pore size should be as close as possible to the analyte of interest, with a preference for thin membranes [7] so as to maximize the signal and the spatial resolution of the nanopore.

In the case of nucleic acid sequencing, for example, it should be possible to read the sequence of individual bases in a DNA molecule as minute variations in the current signal [13], since each DNA base has a unique geometry. In practice, a combination of background noise and signals from neighboring bases prevents the collection of sufficient ions per base, largely due to the rapid passage of DNA through thin solid-state nanopores ( >10 bp/μs). By prolonging the time that a molecule spends within the nanopore, more ions per DNA base would be detected,

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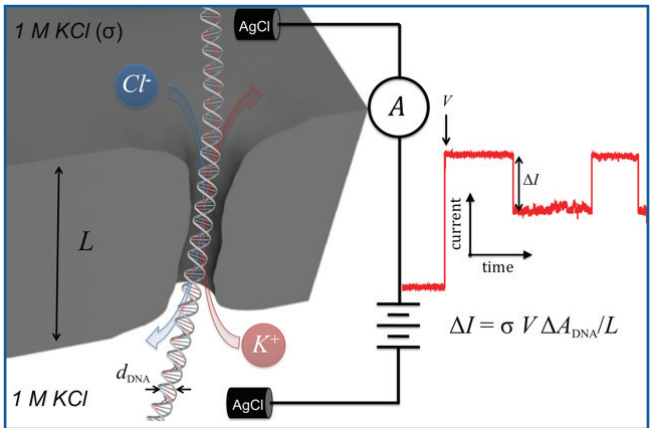


Fig. 1 Schematic representation of a DNA polymer with cross sectional area $\Delta A_{DNA} = \pi d_{DNA}^2/4$, where $d_{DNA} \approx 2.2$ nm, translocating through a 3 nm pore in a 10 nm thick membrane ($L$) immersed in 1M KCl, with conductivity σ under an applied voltage $V$.

thus enabling sequence detection. A number of other applications also stand to benefit from prolonged passage times and the resulting enhanced discrimination ability needed for the multiplexed detection of biomarkers. By modifying the nanopore, its environment, or the analyte itself, researchers have reduced the speed of translocations by nearly 10-fold [14]. Despite this success in reduced passage speed, the propagation dynamics of polymers through pores (i.e., inter- and intramolecular velocity fluctuations) remain relatively unexplored experimentally. Simulations have suggested that the wide distribution observed for the translocation velocities for identical molecules can be attributed to drag-induced velocity fluctuations [15], and a recent experimental study mapping coarse velocity profiles observed that polymers accelerate at the end of the translocation process [16]. In light of these studies, it is evident that controlling passage speed and motion is an active area of research and that a more detailed velocity transfer function is still required to accurately convert temporal signals into positional information.

Nanopore analysis generally requires hundreds or thousands of single-molecule events to build reliable statistics. Enhancement of the typical ssNP capture rate (~$1 s^{-1} nM^{-1}$) is vital for applications requiring timely detection, particularly of low abundance targets. However, few experimental studies have investigated in detail the two-step capture mechanism of charged polymers by ssNP [17,18], in which a molecule diffuses from bulk solution toward a point near the nanopore mouth where field-driven funneling takes over [19]. The characteristic length scale at which the molecule's motion crosses over from almost purely diffusive to drift-dominated motion is called the capture radius — i.e., the "event horizon" (the point of no return) to express the fact that molecules cannot escape and are translocated through the nanopore. For a given applied voltage $V$ and a molecular electrophoretic mobility μ, there exists a region of nonzero electric field outside the pore that defines the capture radius as $r^* = \frac{d_{pore}^2 \mu V}{8LD}$, which is typically on the order of a few microns. Molecular arrival in this capture radius is typically dictated by the diffusion constant $D$ and is thus linear in analyte concentration. However, for low voltages or short DNA fragments, the electrical pulling force barely surpasses the entropic barrier to molecular translocation. Thus, the capture rate shifts from a linear dependence with voltage to an exponential one, dominated by barrier crossing rate as opposed to arrival rate in the capture radius. In this entropic regime, molecular size and conformation also become important. Large pores offer higher capture rate, but their reduced sensitivity means that alternative rate-maximizing approaches are preferred. Alternatives include the use of a salt concentration gradient across the pore, which has been shown to enhance the capture rate by as much as 20 [17], and dielectrophoretic focusing to preconcentrate DNA in the vicinity of the nanopore detector [20]. Despite these developments, many aspects of the nanopore capture process have yet to be investigated in a systematic way, since seemingly identical pores often behave drastically differently.

**Challenges**

Despite intense research efforts and the many exciting developments described above, a number of hurdles remain for nanopore-based technologies to realize their full potential in life science and medical diagnostic applications. Overall, the field is facing three major technical challenges: (*i*) specificity of the signal; (*ii*) control of speed during biomolecular passage through the nanopore; and (*iii*) fabrication of solid-state nanopores in a low-cost, scalable way that is compatible with manufacturing. In our group, we believe that answers to these obstacles are, in one way or another, tied to the technique used to fabricate solid-state nanopores.

**State-of-the-art in ssNP Fabrication**

The desired diameter of a nanopore depends strongly on the application of interest, but should generally match that of the analyte (1.5-2 nm for ssDNA detection and sequencing; 2.5-5 nm for short dsDNA and RNA markers; 5-20 nm for long DNA fragments, genomic screening and protein analysis). Nearly all applications require stable, accurately sized pores in mechanically robust membranes, with thinner membranes favored due to the corresponding increase in ionic current signal [7] and spatial resolution. Until recently, state-of-the-art techniques for achieving such features employed a focused beam of high-energy particles to drill a hole in a ~20-50 nm thick solid-state membrane, followed by a manual-shaping step with a defocused beam to achieve the desired size. The typical techniques – transmission electron microscope (TEM), ion sculpting apparatus, or helium ion microscope (HIM) – involve very expensive instruments (>$1M) that are located in dedicated facilities and call for extensive training and highly qualified personnel for their operation. Further, these techniques require vacuum, and the resulting nanopores must undergo cleaning and wetting steps in order to facilitate immersion in liquid

for sensing. Such time-consuming preparation steps significantly reduce yield and reliability, since pores are frequently damaged during one of many handling steps (especially in sub-30-nm thick membranes) and present high noise levels associated with poor wetting, or size changes due to harsh chemical treatment.

**The Invention, Nanopore Fabrication by Controlled Breakdown (CBD)**

We have invented a simple, rapid, and cost-effective fabrication method capable of creating individual solid-state nanopores directly in a neutral KCl solution. The method relies on application of a sustained electric field across an insulating membrane in solution near its dielectric breakdown strength (~0.5-1 V/nm) by applying a relatively low voltage ( $<$10V across ~10 nm membranes), which induces a localized leakage current through the otherwise insulating membrane. By monitoring the resulting sustained leakage (tunnelling) current, the fabrication of a single nanopore is detected as an abrupt increase in the current, which we attribute to the onset of ionic current. Control of nanopore size in thin membranes is obtained through feedback control used to terminate the application of the electric potential and to limit the damage to the membrane after the initial dielectric breakdown event. The equipment required for fabricating ssNP by CBD is a simple computer-controlled current amplifier circuit to monitor the current and control the electric field strength. Figure 2 highlights the dramatic difference in complexity and cost between CBD and TEM-based nanopore fabrication methods.

The CBD process itself is based on the physics of dielectric breakdown, a phenomenon which has been extensively studied in the context of dry-state semiconductor devices [21]. The underlying kinetics of the process are only partially understood and are theoretically quite complex. Qualitatively, the breakdown process proceeds as follows: as a high electric field is applied to a material near its dielectric strength, the material is no longer fully insulating and will support a leakage current. This leakage current induces damage to the material, in the form of localized charge traps between which electrons or holes can tunnel if they are sufficiently close together. Once a path of these traps exists which electrically connects both sides of the dielectric material, a highly conductive path is formed with a diameter on the scale of ~1 nm, which will sustain a sufficiently large current to locally damage the material. Such percolation-based models of dielectric breakdown predict a Weibull distribution of time-to-breakdown, a feature which we have observed experimentally [22]. In the case of wet breakdown, the kinetics are also influenced heavily by the nature of the ionic solution used to carry the electrical current, being particularly sensitive to the pH, indicating that the electrochemistry at the membrane/solution interface also plays an intrinsic role in the breakdown process. In semiconductor devices, this damage is catastrophic and generally results in the destruction of the device under test, and as such is the eventual failure mode

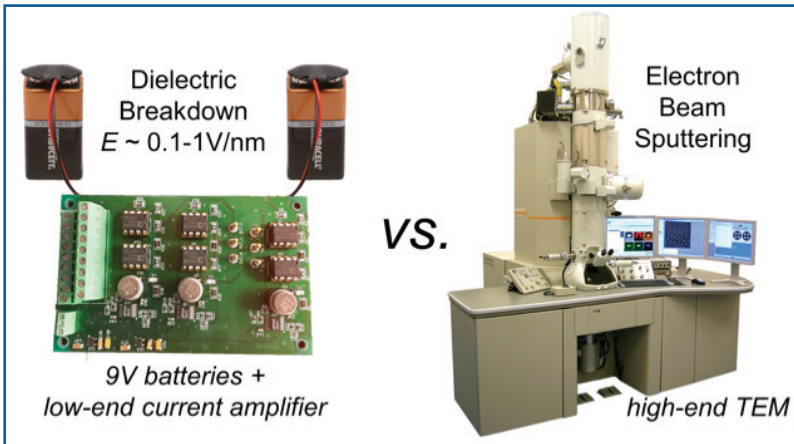


Fig. 2 Nanopore fabrication by controlled breakdown (CBD) only requires application of a relatively low voltage ~10V to generate a high electric field in ~10 nm thick membranes. A simple battery-powered current amplifier circuit controlled by a computer can be used to fabricate ssNP. Note that the circuit shown is capable of forming 3 nanopores on separate membranes in parallel, and the circuitry can easily be scaled up. The alternative method requires a ~$1M, field-emission transmission electron microscope, and can only make one pore at a time.

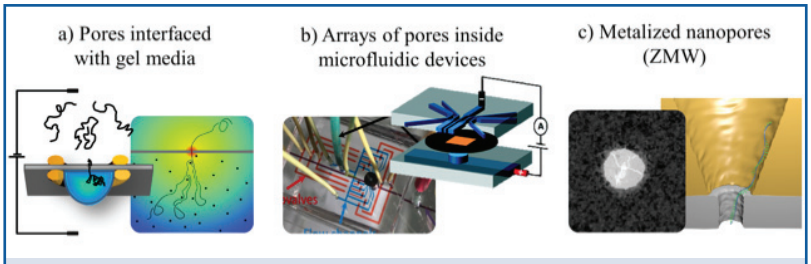


Fig. 3 Examples of research projects. a) A nanopore is interfaced with a gel medium (agarose or acrylamide) on one side of the membrane to reduce the translocation speed during passage (in collaboration with Prof. Gary Slater, Physics, uOttawa); b) a Si chip containing a single free-standing membrane is encapsulated within a microfluidic chip enabling the fabrication of an array of individually addressable nanopores for lab-on-a-chip applications (in collaboration with Prof. Michel Godin, Physics, uOttawa); c) a nanopore is opened in a metalized membrane by CBD thus forming a pore inside a zero-mode waveguide for improved optical detection.

for most computing hardware. For this reason, most of the research on the subject has been performed with the goal of avoiding it entirely. In a liquid environment, however, the damage can be precisely controlled by quickly (within ~0.1s) turning off the voltage once a sudden increase in the leakage current is detected, which signals the formation of the conductive path discussed earlier. Pores formed using CBD are generally on the order of just a few nanometers in diameter, and 2 nm pores can be made reproducibly [23]. Once a pore is formed, its size and electrical characteristics can be precisely tuned using intermediate voltages to controllably enlarge and condition the pore [24]. Because the pore is made in the same solution that is used for single-molecule sensing, it is immediately wetted and ready for experiments. This simple advantage enormously increases the yield compared to particle-beam fabrication techniques, which require multiple handling steps and pre-treatment before wetting is possible.

CBD offers unique opportunities to design and reliably fabricate certain advanced solid-state nanopore devices – such as multichannel [25] or multilayer constructs [26] – that would otherwise be prohibitively challenging, extremely low yield, or simply unfeasible. Over the last few years, we have published articles on the basic principles of the CBD method [27], its reliability and precision [23], and its ability to fabricate pores in multilayered metalized membranes [26]. We have also considered the kinetics of dielectric breakdown in liquid [22] and the propensity for integration into microfluidics for array formation and lab-on-a-chip applications [25]. Further, we have performed nanopore force spectroscopy measurements studying the stretching transition of the B- to the S-form of short dsDNA fragments [23], and worked on strategies to manipulate molecular transport using electrically gated nanopore transistors [28], gold-oligo interactions [26], and nanopores interfaced with gel media [14]. Figure 3 illustrates some of these projects.

## CONCLUSION

The CBD nanopore fabrication method profoundly impacts our capacity to build advanced nanodevices and to perform complex nanopore measurements. These advances bring us closer to delivering real-world solid-state nanopore technologies, with significant implications for the study of fundamental biological processes and for the investigation of biomolecular building blocks like DNA, RNA, and proteins, one molecule at a time.

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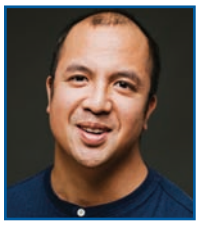
In 2015, the Division of Particle Physics (PPD) created a PhD Thesis Prize competition for best thesis in Subatomic Physics by any student receiving their PhD degree from a Canadian University in the current or prior calendar year. The PPD is pleased to announce that the recipient of the 2016 PPD Thesis Prize is Patrick de Perio. A summary of Dr. de Perio's thesis work appears below.

# NEUTRINOS: PROBES TO THE INNER WORKINGS OF OUR EXISTENCE

BY PATRICK DE PERIO



The understanding of how we came to exist is one of the greatest endeavors of humanity. This involves developing theories that can describe our current state and the entire chain of events from the Big Bang. One question towards this goal is the matter anti-matter asymmetry in the universe. It is very plausible that they were created in equal parts at the Big Bang, and thus should have completely annihilated. However, our existence posits there is some asymmetry in nature that affected the evolution of the early universe.

One candidate theory is leptogenesis, which presumes the existence of heavy neutrinos that violate charge-parity (CP) symmetry, and thus decay to leptons or anti-leptons with different rates. However, such theories must be verified by experimental and observational data. Despite the fact those heavy neutrinos in the early universe are not the light neutrinos we are familiar with today, we must take small steps to understand what is immediately in front of us before we can hope to make the connection to these grand theories.

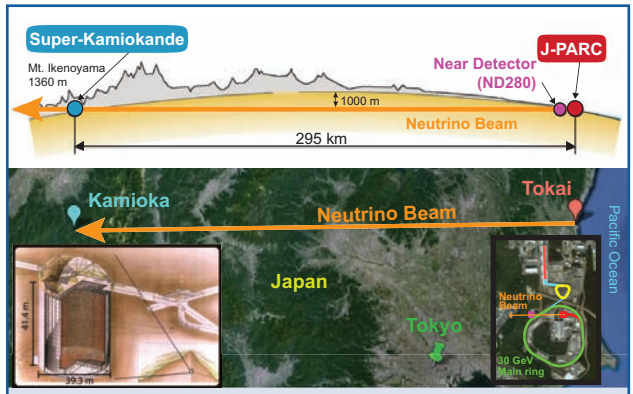



Fig. 1 Overview of the T2K experiment. A neutrino beam is produced at J-PARC (right inset), first measured at a near detector 280 m downstream, then propagates across Japan towards the Super-Kamiokande far detector (left inset) 295 km away.

## THE T2K EXPERIMENT

The T2K (Tokai to Kamioka) long baseline neutrino experiment [1] aims to measure the CP phase parameter in the Pontecorvo-Maki-Nakagawa-Sakata (PMNS) matrix describing neutrino oscillation, a quantum mechanical phenomenon where the three flavours of light neutrinos, electron ($\nu_e$), muon ($\nu_\mu$), and tau ($\nu_\tau$), appear to trade flavours as they propagate. A high intensity $\nu_\mu$ beam is produced from the Japan Proton Accelerator Research Complex (J-PARC), shown in Fig. 1. The beam is directed, first through the Canadian-led near detector for characterizing it, towards the far detector, Super-Kamiokande (SK), the world's largest pure water Cherenkov detector. The neutrinos interact in these detectors producing observable signals as shown in Fig. 2, which we use to infer the flavours of the incident neutrinos and any oscillation.

In order to accurately and precisely measure the parameters in the PMNS matrix, we must understand every aspect of the experiment. My thesis details my contributions to the understanding of the neutrino beam, neutrino interactions, and the SK detector event reconstruction and efficiency. Furthermore, a framework was developed to propagate all of this knowledge, including uncertainties, to a measurement of oscillation parameters.

## MEASURING PROTONS FOR NEUTRINOS

Constraining the neutrino beam direction is necessary to minimize the uncertainty in the oscillation parameters. To this end, an optical transition radiation (OTR) monitor [2], which characterizes the proton beam just prior to collision

**SUMMARY**

**We attempt to build an understanding of nature by precisely measuring neutrino oscillations with the T2K long-baseline neutrino experiment.**

Patrick de Perio <pdeperio@gmail.com>, Columbia University, for the T2K Collaboration

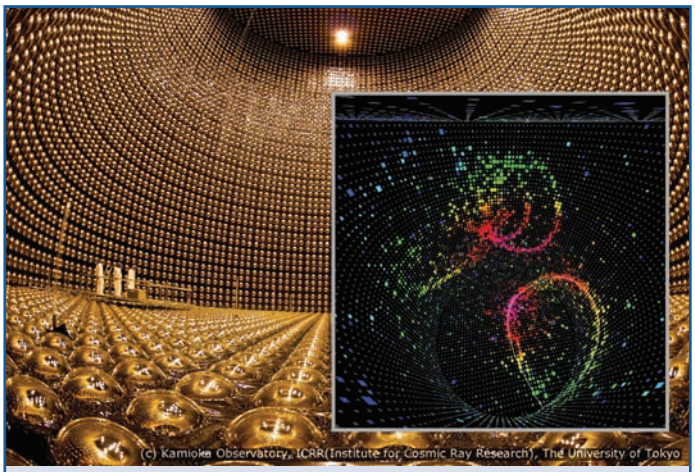



Fig. 2 Inside the Super-Kamiokande detector. During science data taking, the lights are turned off and the tank is filled with 50 kilotonne of ultra-pure water. The >11,000 orb-like objects are photo-multiplier tubes (PMTs) that act as our eyes peering into the center, taking snapshots of Cherenkov light (inset) produced by particles from neutrino interactions (Fig. 3).

with the target for producing the neutrinos, was implemented. The OTR measurements, combined with other beamline monitors, provide the strongest constraint on the prediction of the neutrino beam direction. This is a novel detector with many potential applications for particle beam monitoring in high radiation environments even outside of neutrino physics.

## HOW NEUTRINOS INTERACT

As the neutrinos pass through our detectors they interact with nuclei, producing the lepton and hadron particles that we can measure. Modelling of these interactions is an important and potentially precision-limiting factor in neutrino oscillation experiments. The hadrons are subject to complicated nuclear effects as they traverse the nuclear medium until they escape, as depicted in Fig. 3, after which they can undergo secondary interactions (Sis). Both processes can significantly alter the observable final state and obscure the physics of the initial neutrino interaction, again affecting the oscillation parameter estimation. A single hadron propagation model was implemented in both the neutrino interaction and detector Monte Carlo (MC) simulations, allowing a unified treatment of hadron interactions within the neutrino target nucleus and SIs. Furthermore, a new MC statistical reweighting framework was developed to fit the model to the world's pion-nucleus scattering data, pion photo-production data, and past neutrino experiments, in order to tune and constrain the model parameters, which are propagated to the oscillation analysis.

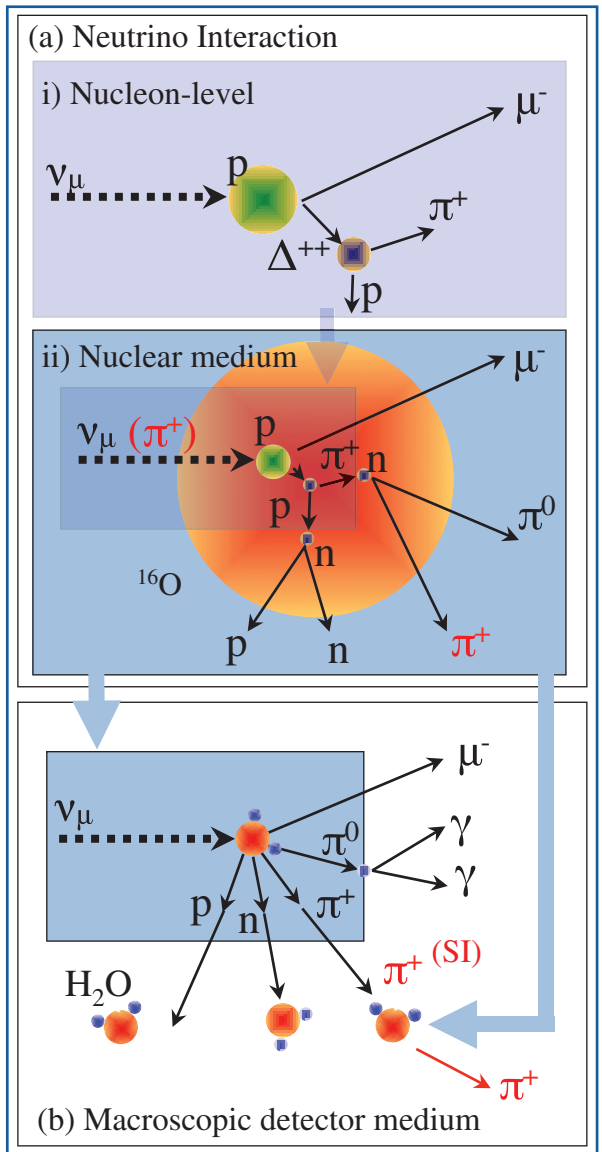



Fig. 3 Cartoon of a neutrino interacting with a proton (a.i) producing a lepton and hadrons that re-interact within the parent nuclear medium (a.ii) prior to escape. The child particles that finally escape the nucleus (b) are what we aim to observe with the detector as in Fig. 2. Furthermore, as particles traverse the detector medium, they may experience secondary interactions (SI) with other nuclei, altering their kinematics or even disappearing completely, which affects the observed light patterns.

## NEUTRINO OSCILLATION ANALYSIS

The SK detector can distinguish $\nu_\mu$ and $\nu_e$ flavours, and we combine both samples to maximize the information for the oscillation parameter measurements. A new Markov Chain MC statistical analysis of the SK atmospheric neutrino data was developed to estimate the detector efficiency systematic errors,

correlated between the two samples. The T2K oscillation analysis combines multiple neutrino oscillation channels while fully treating the correlations in every systematic error source, including the neutrino beam, neutrino and hadron interactions, and detector efficiencies. This spirit of explicitly and precisely propagating all our knowledge resulted in one of the first hints of non-zero CP violation [3] in the lepton sector of particle physics, bringing us one small step closer to a grander understanding of the universe.

This work has been supported by the Natural Sciences and Engineering Research Council, Ontario Graduate Scholarships, the National Research Council, and the Japan Society for the Promotion of Science.

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# Canadian Conference for Undergraduate Women in Physics (CCUWiP)

by Brigitte Vachon

The goal of the Canadian Conference for Undergraduate Women in Physics (CCUWiP) is to encourage undergraduate women and minorities to continue their education in physics by providing them with an opportunity

- to experience a professional conference,
- to receive information about graduate school, undergraduate research opportunities and professions in physics,
- to have access to a network of women in physics of all ages with whom they can share experiences, advices and ideas.

The Canadian conference series is modelled after the successful APS[1] CUWiP [1] conferences held annually all over the United States. The Canadian conference is held over three days, typically during the second weekend of January, which allows undergraduate students to travel and attend the conference without excessive disruptions in their studies. The CCUWiP is held at a different university campus every year. It is primarily intended to be a regional conference such that participants can travel to the conference by car, bus or train. The size of the conference is kept around approximately 100 participants to ensure ample opportunities for participants to meet and interact with each other. The program typically includes research talks by faculty members, presentations from professionals outside academia who have a physics education, panel discussions (e.g., on topics such as graduate school, careers, and research opportunities), presentations and discussions about women in physics, student research talks and posters, and laboratory tours. One of the unique features of the CCUWiP is that it supports the entire cost[2] of students participating in the conference. Participants are responsible for their travelling costs to the conference (e.g., by car, bus, train) but once at the conference location, all accommodations and catering costs are covered by the conference organization. Some travel grants are also made available in support of students with financial needs. This is a very important aspect of what the CCUWiP is about, since it ensures that any undergraduate student can participate in the conference, and not only those undergraduates who have participated in a summer research project and have secured funds through their supervisors to pay for their participation in the conference. Financial support is secured through fund raising efforts undertaken by the conference local organizing committee. The local organizing committee is typically entirely composed of undergraduate students who are driving the organizational efforts at the grass-root level, with some guidance provided by faculty member(s) and some departmental administrative assistance. Given this particular funding model, the total number of participants is limited by the conference budget. An application process is therefore in place to select, if needed, conference participants. Interested students must apply to participate in the conference in the Fall. If more applications are received than the capacity of the conference, then participants are selected based on their degree year, university, and one-paragraph justification of why they would like to participate in the conference. If required, preference is given to senior undergraduate students under the assumption that a first year student will have an opportunity to apply again next year. Participants are also chosen in such a way as to ensure representation from as many institutions as possible thereby maximizing the reach of the conference and cross-institutional networking opportunities for the participants.

The impact of the conference series is monitored through pre- and post-conference surveys completed by participants. This information is useful in determining to what extend some aspects of the conference were effective in

**Summary**

**The fourth edition of the Canadian Conference for Undergraduate Women in Physics (CCUWiP) series was held at McMaster University on 13-15 January 2017. The goals of this conference series and some of its unique aspects are described. A report on this year's conference along with historical statistics from past conferences held in Canada are presented. On-going initiatives in support for the continuity of this conference series are summarized.**

1. American Physical Society.
2. There is a $35 conference registration fee to minimize the number of "no-show".

Brigitte Vachon, <Brigitte.Vachon@McGill.ca>, McGill University (on behalf of members of the CCUWiP local organizing committees)

encouraging women to pursue their physics education, and what aspects could be further improved.

The first conference in this series was held at McGill University in 2014 [2], followed by conferences held at Laval University in 2015 [3], and Dalhousie in 2016 [4].

## 2017 CCUWIP AT MCMASTER UNIVERSITY

This year's CCUWiP conference was held at McMaster University on 13-15 January 2017 [5].

A total of 132 students from 30 different institutions participated in the conference, which received a record breaking 168 applications this year. The break down of participants per institution is provided in Table 1, along with data for earlier conferences. A few pictures of this year's conference are also shown in Fig. 1.

**TABLE 1**
NUMBER OF CCUWiP CONFERENCE PARTICIPANTS PER INSTITUTE AND TOTAL NUMBER OF APPLICATIONS RECEIVED SINCE 2014.

| | 2014 McGill | 2015 Laval | 2016 Dalhousie | 2017 McMaster |
|---|---|---|---|---|
| Acadia | - | - | 4 | 1 |
| Calgary | - | - | - | 4 |
| Carleton | 2 | - | 2 | 8 |
| CHC | - | - | - | 1 |
| Concordia | - | - | - | 1 |
| Dalhousie | - | 4 | 9 | - |
| Guelph | - | 3 | - | 3 |
| Laurentian | - | 6 | 1 | 2 |
| Laval | 7 | 12 | 3 | 4 |
| McGill | 10 | 7 | 6 | 11 |
| McMaster | 7 | 10 | 3 | 18 |
| Memorial | - | - | - | 1 |
| Moncton | - | - | - | 1 |
| Montreal | 2 | 3 | 2 | 1 |
| Mt Alison | - | 3 | 3 | - |
| Okanagan | - | - | 1 | - |
| Ottawa | - | - | - | 2 |
| Queen's | 8 | 8 | 2 | 6 |
| RMC | - | 2 | 1 | 1 |
| Ryerson | - | - | 2 | 8 |
| Saskatchewan | - | - | 3 | - |
| Sherbrooke | 2 | - | - | 6 |
| STFX | - | - | 3 | 1 |
| St. Mary's | - | - | 9 | 1 |
| SFU | - | - | - | 2 |
| Toronto | 5 | 4 | 6 | 11 |
| Trent | 4 | 2 | - | 5 |
| UBC | - | - | 2 | 1 |
| UNB | - | - | 4 | - |
| UOIT | 2 | - | 2 | 7 |
| UPEI | - | - | 3 | - |
| UWO | - | - | - | 2 |
| Waterloo | 2 | 4 | 4 | 14 |
| Western | - | 1 | - | 3 |
| WLU | - | - | - | 1 |
| York | - | 6 | 3 | 4 |
| Participants | 51 | 75 | 78 | 132 |
| Applications | 56 | 110 | 94 | 168 |

The following acronyms are used in the table: College of the Holy Cross (CHC), Royal Military College (RMC), Saint Francis Xavier (STFX), Simon Fraser University (SFU), University of British Columbia (UBC), University of New Brunswick (UNB), University of Ontario Institute of Technology (UOIT), University of Prince Edward Island (UPEI), Universtiy of Western Ontario (UWO), Wilfrid Laurier University (WLU).

Some highlights of this year's conference are briefly summarized here.

For the first time, the CCUWiP conference participated in the live US-wide broadcast of this year's keynote speaker, Prof. Nergis Mavalvala from MIT, who provided a compelling account of the recent discovery of gravitational waves.

A more general emphasis on diversity issues in physics, not only addressing women representation, was also adopted by this year's conference through its workshop/panel offerings and organizational aspects geared at providing an all inclusive environment. This was positively commented on by several participants in the post-conference survey.

Fig. 1 Selected pictures from the 2017 CCUWiP Conference held at McMaster University.

This year's conference also offered a wide range of mini-workshops on diverse topics such as Presentation skills, Graduate Studies in Canada and in the US, Mental health, Organization of a Women in Physics club. The conference also organized a presentation on physics jobs in the industry and a facilitated discussion on the issue of implicit bias.

The analysis of the post-conference survey results shows some interesting trends. Participants reported generally feeling more like a physicist, more part of the physics community, and less isolated from the community after the conference. These results suggest that the conference was successful in creating a supportive and welcoming community for women and students from other minorities studying physics. Participants also reported after the conference feeling more strongly that others saw them as an exemplary student, suggesting they have developed a different attitude towards themselves. One issue to be improved upon is in regards to the apparent increase in participants believing that their successes are due to luck. Despite efforts from the local organizing committee to encourage speakers and panelists to emphasize what they did to get where they are, even senior faculty members will too often attribute their success disproportionally to luck. Future conferences will aim at dispelling this strongly held belief and try to further emphasize the ability of individuals to create for themselves interesting and fulfilling professional opportunities.

## 2018 AND BEYOND

The next Canadian CUWiP conference will be held at Queen's University on 12-14 January 2018. Announcements and opportunities to advertise this conference at each institutions will be made available in the coming months. Institutions are strongly encouraged to already plan their participation in the conference graduate fair.

Host site applications for the 2019 conference will be accepted until 1st November 2017. Interested groups are encouraged to contact Brigitte.Vachon@McGill.ca for more information. The organization of this conference is a particularly rewarding experience and one that provides undergraduate students with opportunities to develop various highly-sought professional skills.

Based on the overwhelmingly positive feedback received, the CCUWiP conference will continue to strive to set the highest standards of accommodation in order to be accessible to all participants regardless of their socioeconomic status, disability, religion, gender or other aspects of their identity.

Various initiatives are also being currently undertaken in order to ensure continuity in this successful and growing conference series in Canada. These include the ongoing development of a partnership with CAP and APS in the US. The APS coordinates a similar set of conferences held annually, and has an established national coordination structure and dedicated resources. Opportunities for long-term technical support (e.g., web application/registration, online payment system,

website hosting) are also being explored to facilitate the organization of this conference series. In Canada, there exists the capacity to simultaneously host up to two CCUWiP conferences, one in "eastern" and one in "western" Canada; or having one conference held annually that would alternate through different regions of Canada. Opportunities for securing some level of stable funding, possibly from private foundations or national granting councils (e.g., NSERC), continue to be sought.

## SUMMARY

The Canadian Conference for Undergraduate Women in Physics (CCUWiP) is a conference series aimed at encouraging women and minorities to pursue their physics education and inform them of the value of this education. Based on conference statistics and data gathered through post-conference surveys, there is a clear demand in Canada for this conference series. The fourth edition in the Canadian conference series was held at McMaster University on 13-15 January 2017, and the next conference will be hosted by Queen's University on 12-14 January 2018. Faculty members around Canada are encouraged to advertise this conference to their undergraduate students and consider acting has mentors for the organization of this conference at their institute. Various initiatives are being currently undertaken in order to ensure the continuity of this successful and growing conference series in Canada. Interested individuals are encouraged to contact Brigitte.Vachon@McGill.ca for more information.

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# GAINING PRACTICAL EXPERIENCE WITH PHYSICS-BASED APPROACHES TO THE MICRO- AND NANOSCALE WORLD OF BIOLOGY

BY NANCY R. FORDE

**SUMMARY**

**As the field of Biophysics has grown in popularity, Physics departments have been adapting traditional course offerings to involve more biological content and introducing courses focused on Physics at its intersection with Biology (e.g., ref. [1]).**

In the last few years, new textbooks focussed on Biophysics have been developed, making it easier for departments to introduce Biophysics lecture courses for their undergraduate students. With a few notable exceptions (e.g., [2] and [3]), however, there are few text-based resources available that describe practical approaches to exploring the concepts, such as computational or laboratory-based activities. Some individual examples of modules can also be found in the *American Journal of Physics* [4, 5] or in other sources [6, 7].

In this *Education Corner* article, we present highlights of pedagogical activities that tie in with the theme of this issue "Nanoscale Approaches to Biological Systems". They are organized approximately by topic, and have been developed by faculty across Canada for application in their teaching to different target audiences. Stand-alone practical courses in Biophysics (such as offered at McMaster University and Simon Fraser University) are the exception, but many offer computational or experimental biophysics modules as part of other courses. The purpose of this article is to present an overview of some available activities, with the aim of providing a useful starting point and resource for others in the Canadian Physics community who are interested in implementing or adapting these modules in their teaching.

Additionally, we hope that the content and contacts within this *Education Corner* article may serve as a starting point for those interested in establishing a "community of practice" in Biological Physics Education in Canada. Contact information for each of the module developers is provided, and we encourage interested readers to connect and help grow this Biological Physics teaching community.

## DIFFRACTION

**Experimentally reproducing DNA's "X" diffraction pattern using springs and laser pointers** – Cécile Fradin, McMaster University. fradin@physics.mcmaster.ca

Developed for a second-year Biophysics course; takes two hours for the experiment plus analysis time.

The structure of DNA was famously solved after Rosalind Franklin obtained an X-shaped diffraction pattern from DNA fibers. She immediately recognized (as did Francis Crick when he later gained access to her data) that the X-shape meant that DNA had a helical structure. A very simple experiment, involving small springs and laser pointers, can demonstrate that helices indeed give X-shape diffraction patterns.

This lab is based on a publication by Braun *et al.* [8], in which students use a simple optical set-up to enlarge the beam of a small laser pointer, and obtain a diffraction pattern from small metallic springs. Proper collimation of the laser beam and proper positioning of the spring ensure the observation of a beautiful X-shaped diffraction pattern. Students can use their phone to take a picture of the diffraction pattern and perform a quantitative analysis of its features. They can also use a microscope equipped with a USB camera and calibration slide to take a real space image of the spring.

Beyond explaining the relationship between a helical structure in real space and an X-shaped pattern in reciprocal space, this lab allows the introduction or illustration of some simple diffraction principles, such as the fact that diffraction informs us about the repetitive features of an object, the relationship between the size of a feature of the object and the size of the corresponding peaks in the diffraction pattern, and the relationship between wavelength and resolution. An associated tutorial done in class in parallel with this experimental module introduces a

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quantitative analysis of DNA X-shaped diffraction patterns that can build on these simple principles.

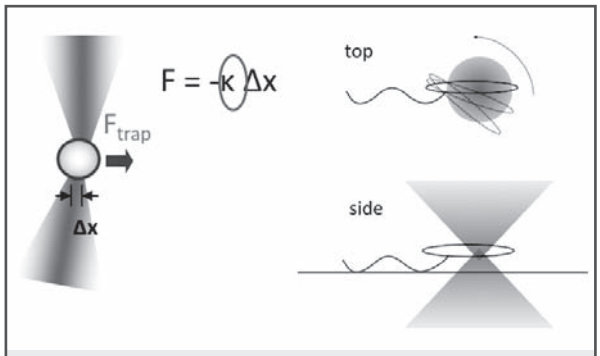


Fig. 1 Optical trapping experiments implemented in the Biological Physics lab course at SFU include using power spectral analysis to calibrate the optical trap stiffness (left) and determining the rotation rate of E. coli flagella as the bacterium rotates through a weak optical trap (right). At the right, the side view shows a snapshot of the bacterium body in the optical trap and its flagellum attached to the surface of the slide. The top view is a schematic illustration of the bacterium periodically moving through the trap as its flagellum rotates the cell body [4].

## NANO- AND MICRO-SCALE MOTION: DIFFUSION AND DIRECTED MOTION

Many modules have been developed, utilizing a wide variety of different approaches, in which students observe and/or simulate diffusive motion and learn how to quantify it. Some of the modules extend to contrast diffusion with biased motion, quantitatively characterizing the different response for each.

**Experimentally distinguishing Brownian from directed motion at the microscale** – Nancy Forde and David Boal, Simon Fraser University. nforde@sfu.ca

Developed for a fourth-year Biological Physics laboratory course and easily adapted for lower levels; takes 2 × 3 hour lab periods.

Surrounded by a fluid in thermal equilibrium, all cells move in response to random interactions with their environment according to Brownian motion. Some cells are also capable of self-propulsion, perhaps by swimming as driven by their flagella or cilia, or by pushing their way past other cells, changing shape as needed. Bacteria may swim in search of food sources, while the macrophages of our bodies may hunt down and swallow invading cells that could be a threat to our health. Such motion can be studied quantitatively using an optical microscope and a CCD camera.

In this module, students learn to operate a microscope in brightfield mode and capture images of diffusing particles and of swimming bacteria. By tracking their positions over time, students distinguish between random diffusion ($\langle r^2 \rangle \propto t$) and directed motion ($\langle r^2 \rangle \propto t^2$). Through knowledge of the Stokes-Einstein relation, students can also compare their experimental estimate of bead size with that provided by the manufacturer.

A related experimental module has been developed by Cecile Fradin at McMaster University, which involves reproducing the Perrin tracking experiment that quantitatively validated Einstein's predictions of random motion.

**Simulating fluorescence images of diffusing molecules and performing image correlation analysis** – Albert Kamanzi, Simon Sehayek and Sabrina Leslie, McGill University. sabrina.leslie@mcgill.ca

Developed for 3rd year students, but can be adapted for lower levels; takes about 7 hours of lectures, plus 3 hours of tutorials for helping students; plus 10-20 hours for completion.

Image correlation analysis is a powerful technique that is used to measure the diffusion coefficients and interaction strengths of molecules in solution. This module begins by instructing students in basic programming skills using Matlab. The students simulate random walks, and gradually convert these simulations to create artificial fluorescence images of diffusing molecules. It introduces technical aspects of fluorescence imaging, such as the finite point-spread function associated with the diffraction limit. Ultimately, the module guides students to perform correlation analysis on their simulated images, from which they can extract biophysical information such as diffusion coefficients, and furthermore understand the impact of measurement settings, such as finite exposure time and noise, on results.

Specific tasks in the modules are the following:

1. Generating symmetric and asymmetric random walks by using Matlab's normally distributed random number function. Using plotting tools to map out trajectories in space and time.
2. Using scatter plots and histograms to analyse the distribution of the end displacements, over several random walks.
3. Estimating the number of particles found in a box of given volume. Finding the one-dimensional expression for the time-correlation function. Finding the characteristic diffusion coefficient from the correlation function.
4. Computing and plotting the correlation function of the generated data, as a function of simulated experimental systematics such as finite exposure and noise, and comparing results to the theoretical expression of the same function.

**Fluorescence Imaging and Diffusion** – Carl Hansen, University of British Columbia. chansen@phas.ubc.ca

Developed for a second-year Physics lab course; performed in stages from prescribed to open-ended over six weeks of 3-hour lab periods.

Students assemble a basic fluorescence microscope and use it to observe both diffusion and chemotaxis. After first assembling a fluorescence microscope from components, students use it to explore diffusion in a concentration gradient of dye molecules created in a microfluidic device. They then explore diffusion of fluorescent beads by tracking the position of beads over time, and finally explore diffusion of *E. coli* bacteria.

**Fluorescence correlation spectroscopy to investigate DNA diffusion** – John Bechhoefer and Nancy Forde, Simon Fraser University. johnb@sfu.ca, nforde@sfu.ca

Developed for a fourth-year Biological Physics laboratory course; takes $3 \times 3$ hour lab periods plus analysis time.

Fluorescence correlation spectroscopy measures the intensity fluctuations of a fluorescently labeled object as it diffuses (and, possibly, drifts) through the focal region of a tightly focused laser beam. It allows single-molecule measurements of diffusion and drift velocity. In advanced applications, one can resolve populations of different classes of objects and changes in properties induced, for example, by ligand binding, and even the motion of single dye molecules.

Recent developments in optics, lasers, photon-detection, counters, and software make do-it-yourself instruments feasible for undergraduate and graduate laboratory courses. In the module developed at SFU, students start from basic exercises that utilize a chopper wheel to temporally modulate laser signal, to develop intuition for the meaning of a correlation function. They then progress to measurements of sizes of fluorescent beads and DNA molecules.

**Simulating the chemotactic motility of *E. coli* bacteria** – Cécile Fradin & Paul Higgs, McMaster University. fradin@physics.mcmaster.ca, higgsp@mcmaster.ca

Developed for a second-year Biophysics course; takes about 4 hours of class time, plus 10-20 hours for completion, depending on programming experience.

*E. coli* are micron-sized bacteria subject to thermal noise. To swim in this environment, they utilize rotation of their flagella (corkscrew-shaped tails). Switching between counterclockwise and clockwise rotation of the flagella controls whether the bacteria swim in a directed fashion or "tumble" randomly.

In this module, students learn some simple techniques for computer simulation that can be applied to problems in physics and biology. Students practice writing their own programs and think about the way a complex real-world problem can be turned into a set of rules that is simple enough for a computational model. These exercises make use of the Netlogo programming environment, which can be downloaded for free and is simple to install on a personal computer. The examples relate to the swimming dynamics of bacteria:

1. Swimming Bacteria - trajectories of bacteria subject to thermal noise
2. Chemotactic Bacteria - switching between swimming and tumbling motion can lead to chemotaxis
3. Foraging strategies - evolution of a chemotactic response is an effective evolutionary strategy in a patchy environment

An experimental module to determine the frequency of *E. coli* flagellar rotation using optical trapping of genetically modified *E. coli*. [4] has been implemented at Simon Fraser University.

## MOTION IN EXTERNAL FIELDS

**Non-linear Electrophoresis** – Andre Marziali, University of British Columbia. andre@phas.ubc.ca

Developed for a second-year Physics lab course; performed in stages from prescribed to open-ended over multiple 3-hour lab periods

DNA mobility in a gel is both length and field dependent – in particular, the velocity of long DNA strands is non-linear with electric field $\boldsymbol{E}$. Students are tasked with designing and carrying out an experiment to accurately measure the non-linearity of velocity vs. $\boldsymbol{E}$. for short and long DNA fragments. After completing this, students choose from a variety of experimental questions, and are required to design an experiment that addresses the question of their choice. One example question is to design an experiment that demonstrates the "IZIFE" (Interface Zero Integrated Field Electrophoresis) effect that exploits the non-linearity of DNA velocity with field previously measured to concentrate DNA at a gel-buffer interface. A second example is to explore biased reptation of large molecules at high electric field strengths, where the velocities are expected to be proportional to field squared.

Students explore the research process, including instrumentation development, experiment design, data analysis, and innovation. The lab is intended to mirror a research lab experience in contrast to the traditional undergraduate lab format.

A related experimental module has been developed by Nancy Forde at Simon Fraser University, and involves students preparing DNA from *E. coli*, and investigating how its electrophoretic mobility is influenced by the DNA topology (e.g., supercoiled, relaxed circular or linear) and length.

## DYNAMICS IN LIVING ORGANISMS MODELLED WITH DIFFERENTIAL EQUATIONS

**Mathematically modelling molecular dynamics in living organisms** – Teresa Zulueta-Coarasa and Rodrigo Fernandez-Gonzalez, University of Toronto. rodrigo.fernandez.gonzalez@utoronto.ca

Developed for a third year Engineering Science laboratory course, and easily adapted to lower levels; takes $3 \times 3$ hour lab periods.

The advent of new microscopy modalities, together with advances in genome editing and computer engineering, is providing a highly detailed view of the molecular interactions and dynamics that govern cell behaviour within living animals. However, as we generate and collect massive amounts of new data, our ability to integrate and interpret these data decreases. Computer models are excellent tools to explore and manipulate complex biological systems in a rapid and inexpensive way, and formulate hypothesis about the molecular underpinnings that control system behaviour for further experimental testing.

In this module, students learn how to build mathematical models of molecular dynamics using Matlab. The module is divided into three sections:

1. Introduction to Matlab and the tools that it provides to implement and solve systems of ordinary and partial differential equations. Students are asked to solve simple systems such as the Lorenz Strange Attractor, used to model chaotic systems, or the one-dimensional heat equation, which describes temperature changes over time.
2. Students implement a system of ordinary differential equations to model a signalling network responsible for the wave patterns that travel through colonies of the myxobacterium *Myxococcus xanthus*. *Myxococcus* glide back and forth in an oscillatory pattern governed by a biochemical clock. Students are asked to use their model to reproduce published experimental results, and to extend the model to formulate a novel biological hypothesis about the molecular mechanisms that regulate the wave patterns.
3. Students implement a model based on partial differential equations to investigate dorsal-ventral patterning in embryos of the fruit fly *Drosophila melanogaster*. The dorsal-ventral patterning system specifies cell fates along the back-to-front axis of the animal. As above, students are asked to validate their model by reproducing experimental results, and to extend the model to provide new biological insight.

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# THE WAR ON FACTS

Fake news, an all-too-common and, to me, rather frightening catchphrase these days, has been used repeatedly by the U.S. President and embraced ad nauseum by his entourage in recent months. Sadly, it has also worked its way into the lexicon far beyond the White House. For proof, one need only look at the comments on any online CBC news article involving the political goings-on south of the border.

How could a mere catchphrase be frightening? Because in this case, its use invariably signals an attempt to blur the lines between truth and falsehood, between inconvenient fact and fiction inspired by wishful thinking.

Propaganda has been around since the dawn of civilization. Yet, until recently, disseminating it has been too expensive in terms of both money and effort for it to run rampant. The democratization of communication through the internet has made the distribution of ideas essentially free. Similar observations were made in earlier times, when the printing press and photocopy machine became commonplace. While the benefits of such technological game-changers are undeniable, they are not without danger. Returning to the present, there is no quality control on the internet, so anyone with the will to do so can say almost anything and, with little effort, they can do it in a way that makes their message, no matter how preposterous, appear legitimate to the uncritical eye.

With fake news comes the idea of "alternative facts", harbinger of an all-out war on the truth. The stakes are dangerously high, with the very credibility of the mainstream media and of science and scientists called into question.

News organizations cannot get too much more mainstream than CNN and the BBC; yet both have been called fake news outlets. (Fox, we are told, is not.) The media more generally has been branded "the enemy of the people" by the current U.S. administration.

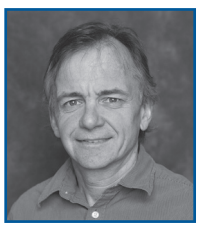
We could perhaps laugh such pronouncements off, yet many of those who voted for the current President believe what he says. When he quotes a discredited Fox News story regarding a preposterous wiretapping claim, they have blind faith in his statement. When he tells us demonstrably true statements by major news outlets are fake news, his denial of the truth becomes their denial of the truth.

More directly related to the CAP, the overwhelming majority of serious scientists who express concern about anthropogenic climate change, calling for immediate action to reduce it, are ignored in favour of more convenient fringe opinions dismissive of any call for action, and indeed dismissive of the very legitimacy of climate change itself, in spite of mountains of evidence to the contrary. It is a hoax put forth by the Chinese, we were told during the American election campaign.

We are relatively lucky in Canada, with a 2017 budget that, while not offering much in terms of new science funding, at least does no harm. Yes, we would have liked to see an increase of funding to the research councils, and, in particular, to the NSERC Discovery envelope[1], but we could have done worse than the status quo[2]. The Government has begun its search for a Chief Science Advisor as announced in the mandate letter to Minister Kirsty Duncan soon after the election, and it maintains its assurance that evidence will be used to formulate government policy. Evidence-based decision making, rather than decision-based evidence making.

Things are not so rosy south of the border. The budget proposal submitted to Congress by the White House on 16 March 2017 includes some very bleak news, including a whopping 31% reduction to the Environmental Protection Agency (now headed by a man hostile to the idea of climate change, and indeed hostile to his own agency!), with smaller but nonetheless damaging cuts to the Department of Education, the National Institutes of Health, NASA, and the Department of Energy's Office of Science. The National Science Foundation did not even garner mention in the budget proposal; one can only guess what that omission implies.

Scientific, academic and professional societies across the US, and to a lesser extent around the world, are justifiably outraged. A compilation of reactions to the U.S. budget proposal, both from politicians and from scientific society



Richard MacKenzie,
<richard.mackenzie@umontreal.ca>,
Département de physique, Université de Montréal, C.P. 6128, Succ. centre-ville, Montréal, Québec H3C 3J7.

1. See the CAP budget submission at https://www.cap.ca/publications/cap-news/cap-makes-submission-house-commons-standing-committee-finance-2018-federal-budget/
2. See the CAP analysis of the budget at http://www.cap.ca/en/news/2017-03-23/2017-federal-budget.

leaders, assembled by the American Institute of Physics makes for an interesting read.[3]

Although the budget itself may not affect Canada and Canadian science directly, it does have an indirect effect across the world. Yet it is the caustic ideas and ideology behind the budget proposal that are very distressing. Ideas know no borders; a war on truth originating in the U.S. is easily exported. And a war on truth has many facets, one of which is a war on the credibility of science, scientific research and indeed scientists themselves. We should all be concerned.

3. https://www.aip.org/fyi/2017/trump-science-budget-reactions-congress-and-scientific-community

## DOCTORATS DÉCERNÉS

# PhD Degrees Awarded in Canadian Universities*
# Doctorats en physique décernés par les universités canadiennes*

### December 2015 to december 2016 / Décembre 2015 à décembre 2016

## Université de Montréal

BELLETÊTE, J., « Représentations et fusion des algèbres de Temperley-Lieb originale et diluée », (Y. Saint Aubin), Septembre 2016, maintenant suit une Post-doctorale à CEA, Saclay, France.

BERTRAND GRENIER, A., « Suivi par élastographie ultrasonore après réparation endovasculaire d'anévrisme aorto-iliaque: étude de faisabilité in vivo », (G. Soulez & G. Cloutier), September 2016, maintenant un Physicien médical (poste temporaire de remplacement) au Centre hospitalier régional de Trois-Rivières, QC, Canada.

CÔTÉ, S., « Développements et applications de méthodes computationnelles pour l'étude de l'agrégation des protéines amyloïdes », (N. Mousseau), March 2016, professeur dans des cégeps de la région de Montréal, QC, Canada.

GÉLINAS, G., « Comprendre et maîtriser le passage de type I a type II de puits quantiques d'In(x)Ga(1-x)As(y)Sb(1-y) sur substrat de GaSb », (R. Leonelli & P. Desjardins), September 2016, now a Research scientist at 5N PLUS Inc., Saint Laurent, QC, Canada.

GIAMMICHELE, N., « Exploitation du potentiel sismique des étoiles naines blanches », (G. Fontaine & P. Brassard), April 2016, maintenant cherche une bourse post-doctorale à l'Université de Toulouse en France.

GUIHARD, M., « Effets des recuits ultra-rapides (10^5 K/s) sur la formation de siliciures métalliques en phase solide », (F. Schiettekatte & S. Roorda), March 2016.

REZASOLTANI, E., "Excitonic Behaviour in Polymeric Semiconductors: The Effect of Morphology and Composition in Hetero-structures", (C. Silva), April 2016, now following a postdoctoral fellowship at the Imperial College London, Kensington, United Kingdom.

*Supplement to the list published in Vol. 73, No. 1 (2017).

*Supplément à la liste publiée dans le Vol. 73, no 1 (2017).

# Melvin A. Preston CD, FRSC (May 28, 1921 - Nov. 1, 2016)

Melvin Alexander Preston, Canadian scientist and academic leader, was born in Toronto, the eldest of six children of G. Alexander Preston and L. Hazel Preston (nee Melvin). His father taught high school mathematics and served as Principal in several Ontario communities. His mother graduated from Queen's University in Kingston, and taught at Ontario Ladies College prior to marriage. Mel grew up, from age 5 to 10, in Shelburne Ont. a village at the junction of Highways 10 and 89. He attended Earl Haig Collegiate in Toronto, and graduated at age 21 with a B.A. in Honours Mathematics and Physics from University of Toronto in May 1942. A point of pride is that in 1942 he became a Putnam Fellow, by placing in the top 5 candidates in the Putnam Prize examination in mathematics. (The Putnam Prize exam is an annual competition open to undergrads in the USA and Canada. Other famous Putnam Fellows include Richard P, Feynman and Ken Wilson (twice).)

Like most young men who graduated in wartime, Mel enlisted in the army, serving as a staff officer in the artillery corps during 1942-45. Among his assignments was to study the use of radar data to direct anti-aircraft fire. He completed his war service with the rank of Captain. Many years later he commanded the Hamilton militia 40 Battery for two years, retiring from the army with rank of Major.

Following the end of the war in 1945, Mel returned to his studies, completing an M.A. at U of T. in 1946, on radioactive alpha-decay. Formally he was supervised by Leopold Infeld, a Pole who had worked with Einstein, but knew little of nuclear physics, so Mel had to learn that from the literature. In 1949 he completed his Ph.D. at University of Birmingham, England under Prof. (later Sir) Rudolf Peierls, a refugee from Germany, like many others, in the 1930's. Peierls had studied with Sommerfeld, and made seminal contributions to both condensed matter and nuclear physics. He was among the first people to reliably estimate the critical mass of Uranium-235 for making an explosion. The Peierls-Fritsch Memorandum to the UK government lead to establishment of the British bomb programme ("Tube Alloys") that was later folded into the US Manhattan Project. In the 1950's, Birmingham had the most prestigious theoretical physics group in England, with a steady stream of visitors. For example, future Nobel Prize winners J.R. Shrieffer and D.J. Thouless held postdoctoral positions in the late 1950's.

Preston went to Birmingham holding the Priestley Fellowship, a scholarship created by Birmingham faculty in gratitude for U of T faculty members taking their children for safekeeping during the war years (including Gaby and Ronnie Peierls). Mel was the first person to hold this fellowship, which was open only to U of T graduates.

From 1949-53 Mel was an Asst. Prof. at U of T. In that period theoretical physics was divided between the Physics Dept. and Applied Maths. Watson, who was Head of Physics, did not support theory. In 1953, Preston was invited by Harry Thode (later the President 1962-1971) to join McMaster in order to build up a theoretical physics group. McMaster was a small Baptist college with enrolment under 1500 students, but Thode had ambitious plans, which were largely realized over the next 17 years. By 1969 the group comprised half a dozen faculty, five PDF's and a dozen graduate students.

In 1962 the theory group consisted of Preston, Sy Vosko (Assoc. Prof.), Doug Twose and Donald Sprung; a couple of PDF's and half a dozen graduate students. It had taken a while to recruit someone in condensed matter physics (Vosko) who would stay. Among people who passed through the theory group, was Rudy Haering, who later became Chair of Physics at Waterloo, then Simon Fraser, and finally UBC. Preston was always generous to the younger faculty members. Initially he controlled all the research funding, and for 20 years we pooled our grant money for maximum effectiveness.

From 1965-71, Preston served as Dean of Graduate Studies at McMaster. This involved him in the process of building up graduate studies across McMaster, and ultimately across Ontario. From 1971-75 he was Executive Vice-Chairman of the Advisory Committee on Academic Planning of the Council of Ontario Universities. In this role he instituted and oversaw a process for assessing the quality of new and existing graduate-level programs across the rapidly expanding Ontario system. I think this is the role he felt was the most important of his career.

From 1975-77 he returned to academic life as Chair of Applied Mathematics at McMaster. This Department belonged to both Science and Engineering, and had three foci: applied analysis, applied statistics, and computer

science. Several members held cross-appointments in Clinical Epidemiology and Biostatistics, in the Faculty of Health Sciences. CE&B played a key role in advancing evidence-based medicine.

From 1977-82 Mel was V-P Academic at U Saskatchewan. In this role he faced unionization of the faculty and was involved in negotiating their first contract. I think he saw this as an important contribution, but it made him few friends. After the VP role ended, he remained at U Sask. until 1986, when he retired and returned to McMaster with an appointment as Prof. Emeritus of Physics. In 1990-94 he supervised his last PhD student (from Libya; Abdalla Ruken, who has made his career in financial services with ScotiaBank.) Preston taught undergrads general relativity until 2004.

Preston was well known for his 1962 monograph "Physics of the Nucleus" (Addison-Wesley) and its revision as "Structure of the Nucleus" (1975) with his best known student Rajat Bhaduri as coauthor. Norman K. Glendenning did an M.Sc. with Mel in his first years at McMaster, then went on to a productive career in Berkeley. Other students made careers in Canada: J. Michael Pearson at U. de Montreal. Subal das Gupta at McGill, David Kiang at Dalhousie, Wytse Van Dijk at Redeemer College in Hamilton, Kailash Kumar in Australia, and Pierre Grange in Strasbourg, France. Mel's students uniformly praised his care and attention as a supervisor.

Mel married three times: Mary Whittaker (1947, d. 1965); Eugene Shearer (1966, d. 1996), and Helen Howard-Lock (1999). He is survived by two sons (Jon and Richard from his first marriage), and numerous family members on the Howard-Lock side.

Donald Sprung
McMaster University

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CAP members are given the first opportunity to request books. For non-members, only those residing in Canada may request a book. Requests from non-members will only be considered one month after the distribution date of the issue of *Physics in Canada* in which the book was published as being available.

The Book Review Editor reserves the right to limit the number of books provided to reviewers each year. He also reserves the right to modify any submitted review for style and clarity. When rewording is required, the Book Review Editor will endeavour to preserve the intended meaning and, in so doing, may find it necessary to consult the reviewer. Reviewers submit a 300-500 word review for publication in PiC and posting on the website; however, they can choose to submit a longer review for the website together with the shorter one for PiC.

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*Si vous voulez faire l'évaluation critique d'un ouvrage, veuillez entrer en contact avec le responsable de la critique de livres, Richard Marchand, en utilisant le formulaire de demande électronique à http://www.cap.ca.*

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# Books Received / *Livres reçus*

The following titles are a sampling of books that have recently been received for review. Readers are invited to write reviews, in English or French, of books of interest to them. Unless otherwise indicated, all prices are in Canadian dollars.

Lists of all books available for review, books out for review and book reviews published since 2011 are available on-line at www.cap.ca (Publications).

In addition to books listed here, readers are invited to consider writing reviews of recent publications, or comparative reviews on books in topics of interest to the physics community. This could include for example, books used for teaching and learning physics, or technical references aimed at professional researchers.

*Les titres suivants sont une sélection des livres reçus récemment aux fins de critique. Nous invitons nos lecteurs à nous soumettre une critique en anglais ou en français, sur les sujets de leur choix. Sauf indication contraire, tous les prix sont en dollars canadiens.*

*Les listes de tous les livres disponibles pour critique, ceux en voie de révision, ainsi que des critiques publiées depuis 2011 sont disponibles sur : www.cap.ca (Publications).*

*En plus des titres mentionnés ci-dessous, les lecteurs sont invités à soumettre des revues sur des ouvrages récents, ou des revues thématiques comparées sur des sujets particuliers. Celles-ci pourraient par exemple porter sur des ouvrages de nature pédagogique, ou des textes de référence destinés à des professionnels.*

### GENERAL LEVEL

**Can the laws of physics be unified?(V)** Paul Langacker, Princeton University Press, 2017; pp. 271; ISBN: 9780691167794; Price: 43.95. (Live: 1)

**Sleeping Beauties in Theoretical Physics [v],** Padmanabhan Thanu, Springer, 2015; pp. 302; ISBN: 978-3-319-13443-7; Price: 81.78.

**The Eternal Darkness: A Personal History of Deep-Sea Exploration (V),** Robert D. Ballard With Will Hively, Princeton University Press, 2017; pp. 408; ISBN: 9780691175621; Price: 28.06.

**The Worst of Times: How Life on Earth Survived Eighty Million Years of Extinctions [v],** Paul B. Wignall, Princeton University Press, 2015; pp. 244; ISBN: 9780691176024; Price: 34.95.

**What Goes Up . . . Gravity and Scientific Method,** Peter Kosso, Cambridge University Press, 2017; pp. 240; ISBN: 978-1107129856; Price: 56.95.

### UNDERGRADUATE LEVEL

**From Photon to Neuron: Light, Imaging, Vision [V],** Philip Nelson, Princeton University Press, 2017; pp. 512; ISBN: 9780691175195; Price: 136.62.

**Modern Elementary Particle Physics: Explaining and Extending the Standard Model (2nd Edition),** Gordon Kane, Cambridge University Press, 2017; pp. 240; ISBN: 9781107165083; Price: 65.50.

**The Standard Model in a Nutshell (V),** Dave Goldberg, Princeton University Press, 2017; pp. 320; ISBN: 9780691167596; Price: 99.78.

## SENIOR LEVEL

**ADVANCES IN DISORDERED SYSTEMS, RANDOM PROCESSES AND SOME APPLICATIONS,** Pierluigi Contucci (Editor) and Cristian Giardin (Editor), Cambridge University Press, 2016; pp. 380; ISBN: 978-1107124103; Price: 153.30.

**APPLICATIONS OF CHALCOGENIDES: S, SE, AND TE [V],** Ahluwalia, Gurinder Kaur (Ed.), Springer, 2017; pp. 461; ISBN: 978-3-319-41188-0; Price: 185.22.

**ECOLOGICAL FORECASTING [V],** Michael C. Dietze, Princeton University Press, 2017; pp. 288; ISBN: 9781400885459; Price: 81.95.

**NATURAL COMPLEXITY: A MODELING HANDBOOK PAUL CHARBONNEAU [V],** Paul Charbonneau, Princeton University Press, 2017; pp. 349; ISBN: 9781400885497; Price: 62.32.

**RAYS, WAVES, AND SCATTERING: TOPICS IN CLASSICAL MATHEMATICAL PHYSICS [V],** John A. Adam, Princeton University Press, 2017; pp. 616; ISBN: 9780691148373; Price: 93.24.

**RELATIVISTIC KINETIC THEORY: WITH APPLICATIONS IN ASTROPHYSICS AND COSMOLOGY,** Gregory V. Vereshchagin and Alexey G. Aksenov, Cambridge University Press, 2017; pp. 334; ISBN: 978-1107048225; Price: 157.99.

**SUPERSYMMETRY, SUPERGRAVITY, AND UNIFICATION (CAMBRIDGE MONOGRAPHS ON MATHEMATICAL PHYSICS),** Pran Nath, Cambridge University Press, 2016; pp. 536; ISBN: 978-0521197021; Price: 94.99.

**SUPERSYMMETRY, SUPERGRAVITY, AND UNIFICATION (CAMBRIDGE MONOGRAPHS ON MATHEMATICAL PHYSICS) - 2ND COPY,** Pran Nath, Cambridge University Press, 2016; pp. 536; ISBN: 978-0521197021; Price: 94.99.

**THE STRUCTURE AND DYNAMICS OF CITIES: URBAN DATA ANALYSIS AND THEORETICAL MODELING,** Marc Barthelemy, Cambridge University Press, 2017; pp. 278; ISBN: 978-1107109179; Price: 94.99.

# BOOK REVIEWS / *CRITIQUES DE LIVRES*

Book reviews for the following books have been received and posted to the Physics in Canada section of the CAP's website: http://www.cap.ca.

*Des revues critiques ont été reçues pour les livres suivants et ont été affichées dans la section "La Physique au Canada" de la page web de l'ACP: http://www.cap.ca.*

**GROUP THEORY IN A NUTSHELL FOR PHYSICISTS** by A. Zee, Princeton University Press, 2016, ISBN: 9780691162690, pp: 632, Price: 125.95$.

After superb books on Quantum Field Theory and on General Relativity, Anthony Zee came out with a new textbook on another subject of high importance in physics: Group Theory. Zee's "Group Theory in a Nutshell for Physicists" is different from most other books on the subject in many aspects. The great clarity of the explanations, the variety of topics covered and the emphasis put on the physics grant it in my opinion the title of "Group theory book that every physicist should read".

The book starts with a review of linear algebra and jumps quickly to the notions of discrete and continuous groups. The highly important representations and character tables are introduced and the relation between group theory and degeneracies in quantum mechanics is explained. The most important part is about the construction of irreducible representations of Lie groups with the tensor and the ladder operators methods, which are used to describe isospin and the eightfold way of particle physics and lead to the classification of Lie algebras using Dynkin diagrams. The author discusses moreover the Lorentz algebra and spinors using representation theory and goes quickly over the conformal algebra and the group theory behind the expansion of the universe. Finally, the road from the Standard Model of particle physics to grand unification is presented.

Like in all of his nutshell books, Zee starts his discussion with the very basic notions and builds towards really advanced topics. Group theory as a mathematical subject requires essentially only linear algebra and every physicist who has some knowledge of quantum mechanics can understand almost all of the physics in the book. Furthermore, even though the applications to more advanced topics can be intimidating for beginners, Zee explains very clearly the minimal knowledge required for the group theoretic applications.

From the point of view of a graduate student, what sets this book apart from the others is the special topics that are covered, mostly in the appendices and in the interludes. I personally really enjoyed reading about the classification of Lie algebras, spinors in general dimension and the conformal algebra. The section on the Lorentz group should in my opinion be read by every QFT student because it is clearer than what is done in most QFT textbook.

Zee's style of writing has always been one of the things that make his textbooks so popular. He writes as if he was giving a lecture to a class instead of writing a book. You know that he thought about the subject a lot and he is not scared of discussing the "stupid" questions. It is also obvious in the text that it is written by a physicist for physicists because the focus is more on intuition and examples than on mathematical rigor.

To summarize, both new and advanced physicists will enjoy this book, but in different ways. Unlike the other nutshell books by Zee, this one contains enough details to teach most readers everything they need to know about the subject. Nonetheless it is really important to do all the exercises and fill the missing steps in the calculations to understand everything. I recommend this book to literally every physics student because we all need to know this useful and beautiful subject.

Yan Gobeil
McGill University

**RELIABILITY IN SCIENTIFIC RESEARCH: IMPROVING THE DEPENDABILITY OF MEASUREMENTS, CALCULATIONS, EQUIPMENT, AND SOFTWARE 1ST EDITION** by I.R. Walker, Cambridge University Press, 2011, pp: 610, ISBN: 978-0-521-85770-3, price: 91.16.

The author's intent in writing this book was to assist academic scientists and engineers to identify potential sources of experimental problems in their laboratory and to provide advice to prevent and deal with such difficulties, should they occur. This 580-page reference summarizes two decades of the author's experience at the Cavendish Laboratory into 14 carefully crafted chapters that are full of advice and best practices. Having used this text in our imaging device development laboratory for

the last year, we can concur that the author has achieved his goal.

Over 80% of the book covers general laboratory situations, which will be of particular value to students and early career experimental researchers, no matter what their field. These chapters cover, the basic principles of reliability, human error, and other general issues; Mathematical calculations; Basic issues concerning hardware systems; Obtaining items from commercial sources; General points regarding the design and construction of apparatus; Mechanical devices and systems; Electronic systems, Interconnecting, wiring, and cabling for electronics; Computer hardware and software, and stored information, and finally, Experimental methods.

Some chapters are however more specific, covering situations that reflect the area of expertise of the author and these, which cover, Vacuum systems leaks and related problems; Vacuum pumps and gauges, and other vacuum system concerns; Cryogenic Systems and Visible and near visible optics, will probably only be of interest to those working with such applications. While my lab has little current need for these sections, the sage advice on detecting vacuum leaks would have been most welcome early in the career of one of us (SP). Irrespective of what your field of research, the extensive (24 page – double column) index, makes it easy to navigate to the section of interest.

The advice provided is clear, concise and practical. It includes, ways to reduce experimental and mathematical errors, precautions to prevent damage and deterioration caused by equipment transport, guidance on when and how to design and construct your own equipment, and how to minimize RF interference, challenges faced when connecting and routing cables, and recommendations for computer hardware and software design and much, much more.

Each chapter concludes with a valuable summary of key points, making it easy to identify and retain relevant information. This is backed up by an extensive list of references, books, and useful websites, which provide more detailed information on topics such as how to eliminate ground loops or how to solder properly. Given the usefulness of these sections, we would like to see a second edition where the key points and references are more readily distinguishable from the rest of the text.

Graduate students in my lab have obtained indispensable guidance from many chapters of this book, and it has become a useful addition to our library. We particularly liked the all too short final chapter, which uses some real-world examples to highlight the pitfalls of experimental research, and of course how to overcome them.

The book could in our view, benefit from some minor improvements. The chapter on Computer Hardware and Software, while valid when it was first published, is showing its age and would benefit from an update. Some sections are too specific and are limited to the experience (and biases) of the author. A second edition that included advice from contributors in other fields would enhance the value of this already helpful book.

The author states that the book would be most useful to young scientists and engineers. However, at a current cost of less than $100, the purchase of this book by Principal Investigators for their laboratory would be a sound investment, given the potential saving of time and money that could be achieved, if the advice in this book is routinely followed.

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**Statistical and Thermal Physics** by Michael J.R. Hoch, Taylor & Francis Group, 2013, pp: 423, ISBN: 9781439850534, price: 104.82.

This book introduces thermodynamics and statistical physics using a unifying approach whereby the macroscopic and microscopic descriptions are presented in a complementary fashion. Concepts are explained using ideal non-interacting spins and ideal gases as models. This combined presentation allows gaining significant insight into classical and statistical thermodynamics. However, the particularities that differentiate the statistics used to describe indistinguishable versus distinguishable particles become less clear for the learning reader.

The first part deals with the microcanonical ensemble description mostly from a classical thermal physics perspective. After the introduction of the first and second law, the notions of microstate and density of states are presented. The specific case of two localized spin systems undergoing thermal interaction is generalized to illustrate the complementary statistical interpretation of entropy. The third law as the temperature approaches zero is explained. Thermodynamics potentials and the Maxwell relations are defined.

The second part begins with a review of probability theory and statistical physics ensembles. After having defined the partition function under the canonical description, the spin system example is used to derive the general relationship between the partition function and the Helmholtz potential. The grand canonical ensemble is introduced with a view to derive the quantum distribution functions. Various applications are discussed. The limiting case corresponding to the classical ideal gas is presented, followed by more advanced topics on non-equilibrium thermodynamics.

The text is very well written using plain language. It is well suited for a senior undergraduate course, with emphasis placed on applied spin systems. The combined use of ideal paramagnets and gases as system models is especially useful in the illustration of classical and statistical thermodynamics processes. Although the author is careful in mentioning the distinguishable nature of the spin particles under consideration, the topic of "Boltzmann statistics" for localised particles is not addressed as a whole. With this approach, it might be more challenging for a student to gain a global understanding of the classical Boltzmann statistics used to describe a system of non-gaseous and weakly interacting particles.

The book thoroughly covers the relevant statistical physics applications which can typically be found in other textbooks. Nevertheless, notions of probability theory and distribution functions are introduced late. A lot of insight can be gained by defining the one particle microstates through the simple binomial counting problem and then, by deriving the most probable distribution function using the method of Lagrange multipliers. After having introduced the partition function under the canonical ensemble approach, the author derives the quantum distribution functions using the grand canonical ensemble. Still, it is a powerful method to obtain the same quantum distributions, but it requires an understanding a priori of the more abstract concepts related to the grand canonical ensemble.

This book offers an original perspective by introducing thermal and statistical physics concepts with ideal spins and gases serving as models, instead of having one section dedicated to systems of distinguishable particles. The content is clearly presented with less emphasis on detailed mathematical manipulations. It should be useful to those interested in the study of paramagnetism.

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*No.1* Neutron beam /
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