Editorial

Getting High on Single Molecule Biophysics

The 5th biennial winter workshop on Single Molecule Biophysics (SMB) was held at the Aspen Center for Physics in Aspen, CO, over the week January of 2-9, 2009. The resort town of Aspen lies in a high mountain valley at 8,000 ft. (2440 m) elevation in the Rockies, surrounded by some of the tallest peaks in Colorado, and the stunning views from its ski slopes can literally take your breath away. But the *cognoscenti* know that Aspen, among its many other claims to fame, has also been a traditional rendezvous point for physicists, who gather from all over the world at the renowned Aspen Center for Physics (ACP) for its scientific programs held in the summer and winter. In a phrase, Aspen is to physics what Woods Hole is to biology, with majestic mountains replacing the serene seashore. Ullr, the Norse god of skiing, smiled once again upon the SMB meeting, because the snow-loving conferees were treated to fresh doses of Colorado's famous powder nearly every day of the week. This made for some fabulous skiing and snowboarding. Perhaps even more fortunately, the near-daily snowstorms abated during the two periods when it really counted, namely, as folks were traveling into Aspen for the start of the conference or leaving — with smiles on their faces! — at the end. It has been my personal pleasure to organize all five of the SMB conferences, which have been held in alternate winters ever since 2001, and therefore to witness first-hand the ascendancy of single molecule biophysics as a scientific discipline. This issue of *Current Pharmaceutical Biotechnology* celebrates recent progress with a special "hot topic" volume, compiled by Editor-in-Chief Zeno Földes-Papp, who also participated in SMB 2009 and co-authored a paper in this issue. In these pages, you will find eleven papers contributed by meeting participants, covering a broad range of subjects. Taken together, they convey the current sense of excitement and ferment in the field, and testify to the stunning progress in single molecule research that's been achieved over the past decade. Today, we routinely carry out experiments on individual biomolecules that were only pipe dreams a scant few years ago.

The field of single molecule biophysics continues to enjoy widespread interdisciplinary interest, solid federal funding support, and strong growth. All in all, 110 participants were accepted to SMB 2009 (drawn from a strong pool of nearly 200 applicants), representing an increase in enrollment of 10% over 2007, despite the weakness in the economy. In fact, enrollment in the SMB conferences has increased steadily every year since these were established a decade ago. For 2009, participation had to be limited for the first time to the number of seats available in the auditorium at the ACP. Over the years, the SMB conference has grown to become the premier meeting in its field, and, as in years past, the get-together was a tremendous success by all accounts. The SMB meetings are distinguished by academic diversity, with participants drawn from a wide variety of sub-disciplines, including theoretical & experimental physics, molecular and structural biology, biochemistry, chemistry, engineering, mathematics, and medicine. More than 50 short platform talks were presented over a five-day period. This year also featured two jam-packed poster sessions, with more than 75 posters. There was excellent international representation, with participants drawn from major universities in North America, Asia, the Middle East, and Europe. Attendees included a carefully balanced mix of established professors, junior faculty, postdoctoral researchers, graduate students, and representatives from national laboratories. Approximately 23% of conferees in 2009 were women or minorities. Financial support for the meeting was raised from both public and private sources. This year, the list of sponsors included Andor Inc., Chroma Inc., Cytokinetics Inc., Hamamatsu Inc., JPK GmBH, Mad City Labs Inc., Nikon Biomedical Inc., Physik Instrumente LLP, Princeton Instruments/Roper Inc., the Royal Society for Chemistry (UK), Spectra-Physics Inc., and Carl Zeiss Inc. Major funding support also came from the National Science Foundation, which helps to underwrite many activities of the ACP. The funding raised was primarily used to defray a portion of the expenses of younger scientists and those participants traveling long distances, however, it proved possible to award at least some level of aid to nearly every one of the participants.

In addition to intense, twice-daily science sessions and two (crowded!) night-time poster sessions, several special events were scheduled to enliven the proceedings. The Reception on Sunday evening featured a performance of live bluegrass music by some very talented Aspen-area musicians, *The Flying Dog Bluegrass Band*. It was my privilege to sit in with the band myself for a few numbers on the five-string banjo, and also to break out my mandolin for an old-time fiddle tune or two. Quite a few of the meeting participants, it transpired, were long-time devotees of bluegrass music—Cees Dekker (Technical University of Delft, Netherlands) even plays in a European bluegrass band! —and some new converts were won over as well.

On Wednesday, as part of its outreach program, the ACP teamed up with a local Aspen organization to host a Physics Café in the mezzanine lobby of Aspen's historic Wheeler Opera House (1889), a beautifully restored Victorian-era theater in the heart of downtown. The Physics Café, which has become something of a local tradition, provides an opportunity for Aspen's local residents to hear firsthand why the scientists have gathered to meet and what the excitement is all about, and to pose any questions that come to mind. These tend to be lively events. This year, the Physics Café featured short presentations by three of our international participants, who courageously proceeded to field some wide-ranging questions: Dr. Christoph Schmidt (Georg-August University, Göttingen, Germany), Dr. Claudia Veigel (National Institute for Medical research, Mill Hill, London, United Kingdom), and Dr. Henrik Flyvbjerg (RISØ National Laboratory, Roskilde, Denmark). The Physics Café was followed by the De Wolf Lecture, held in the main theater and open to the general public. This year, the lecture was delivered by Prof. James A. Spudich (Stanford University), entitled "*Nature's Exquisite Nanomachines: The Dynamic and Varied City Plan of Living Cells*." His talk was well attended, and the local audience seemed fascinated to learn (some, for the very first time) about the amazing array of protein-based machines responsible for so many important processes in life, including molecular motors such as myosin and kinesin. The audience was sufficiently captivated that the question period afterwards had to be extended. I can also report, as meeting organizer, that positive feedback about the De Wolf lecture kept pouring in for the remainder of the week, including kudos offered by professional scientists living in the Aspen area.

Thursday afternoon featured the traditional NASTAR race, which has been a source of friendly competition and bragging rights at the SMB meetings since their inception. NASTAR (an abbreviation for National Standard) is a dual-format giant slalom ski and snowboard race where competitors can qualify for medals based on their best race times, under a handicapping system that pits their results against a set of uniform standards, established nationally by elite racers at the start of each season. Biophysicists, it seems, make for talented skiers! This year, sixty-three meeting participants and their family members produced qualifying times in the NASTAR race (out of roughly 75 participants), winning a record number of medals (4 gold, 12 silver, and 20 bronze), a total that surpassed all other winter workshops held at the ACP. The men's ski race was won by B. Gaub (Max Planck Inst., Munich, Germany), followed by T. Perkins (Univ. Colorado) and A. Grindley (Yale University). The women's race was won by L. Finzi (Emory University), followed by C. Grindley (Yale University) and A. Iwane (Osaka Univ., Japan). The snowboard competition was won by E. Schaeffer (Max Planck Institute, Dresden, Germany), followed by R. Phillips (Calif. Inst. of Technology) and D. Rueda (Wayne State Univ.). An award for Special Merit went to K. Frieda (Stanford Univ.), who ran down the giant slalom course on snowshoes, edging out J. Andrecka (Univ. Munich). Those interested in viewing the race results for the team named "Single Molecule Biophysics" can find these online at: http://www.nastar.com/index.jsp?pagename=raceresults&race=65105&year=2009. Incredibly, the biophysicists managed a team ranking of 529 out of 3,917 teams in the United State for the 2008-2009 ski season (13th percentile); our best showing ever.

The NASTAR race was followed by the Meeting Awards Ceremony & Banquet that same evening, with special prizes and trophies going to all the winners, and also to many of the losers—and with plenty more prizes to go around for all the other meeting participants, as well! The after-dinner ceremony also featured the presentation of the Martin & Beate Block Scholarship, from an endowed fund of the ACP that furnishes a merit-based scholarship to one young scientist selected from each of the (currently five) winter meetings every year. The award for SMB 2009 was personally presented by physicist and long-time Aspenite Martin Block, who was responsible for founding the ACP winter meeting series back in 1985, along with his wife Beate Block (who, at age 83, also managed to snag one of the four gold NASTAR medals in the ski race). Yes, for those of you who may be wondering, they're my parents! This year's Block scholarship was won by Ms. Anna Kochaniak, a graduate student in Antoine van Oijen's lab at Harvard University, for her presentation "*Single-molecule observation of the rotational and translational movement of the PCNA sliding clamp along DNA*." Along with a check for $500, she received a custom-made cube of crystal glass with a three-dimensional molecular structure laser-etched inside, displaying the PCNA clamp protein surrounding the DNA helix.

The eleven papers found in this issue provide a snapshot of what's happening in the field of single molecule biophysics. As is so often the case, progress on the technical side of things is rapidly turned into newfound scientific knowledge. Perhaps fittingly then, around half the contributed papers discuss new developments in biotechnology that are driving single molecule work, while the others present biological results from recent research on single-molecule systems. As the field of single molecule biophysics matures, we're beginning to see more commercial development of apparatus, perhaps signaling the beginning of a trend away from the "roll your own" approach, and one which may make single molecule experiments more accessible those without some background in physics or engineering. The paper by Wozniak and coworkers describes the design and performance characteristics of one commercial system for optical trapping, the JPK NanoTracker, which is (to my knowledge) the first commercial system to come equipped with the ability to sense the position of the trapped object on the nanoscale, using quadrant photodiode detection. As such, it should be applicable to a much wider range of single molecule applications than the commercial trapping systems which came before it. The paper by Jordan & Anthony reviews improved ways to do nanopositioning using a variety of microprocessor-driven piezoelectric devices, an activity that is absolutely central to work with a whole gamut of single-molecule and single-cell approaches, including scanning-force microscopy, ultra-high resolution optical microscopy, single-molecule fluorescence, and optical trapping. Some of the recent progress in single molecule biophysics has come from combining various new imaging and measurement modalities, for example, optical force spectroscopy and single-molecule fluorescence. However, the marriage of these diverse techniques in a single apparatus has posed some serious experimental challenges. For example, the visible light emitted by a single fluorophore attached to a protein of interest is typically about 15 orders of magnitude less than the infrared light required to optically trap that same protein for study, and this can make it very hard to detect fluorescence against the background. Furthermore, the fluorophore can rapidly become extinguished by two-photon or other effects induced by the trapping laser. The paper by Ferrer *et al.* describes a clever approach that can substantially reduce such photobleaching, by rapidly alternating the trapping and fluorescence excitation lasers. Michalet and colleagues review recent progress on the development of a new kind of low light-level camera that they are calling "H33D", a contrived acronym intended to mean "high spatial-resolution, high temporal-resolution, high throughput, three-dimensional detector." The camera is under development by teams of biophysicists and astrophysicists in a collaboration that includes the Lawrence Berkeley and Livermore National Labs; the first prototype was released in 2004. The goal is nothing less than to produce a widefield camera that can not only image at extremely low light levels, but also count and time the individual photons as these arrive at each pixel location, in effect combining the most desirable features of sensitive avalanche photodiodes and modern EM-CCD cameras. They report on recent test results based on recording from single quantum dots. Földes-Papp and colleagues tackle the thorny problem of reducing the background light contribution in single-molecule studies, which has plagued many studies of molecular fluorescence in solution, and particularly in live cells. They demonstrate how optimized time-gating of the fluorescence signal, together with time-correlated, single-photon counting, can be used to substantially boost the experimental signal-to-noise ratio (roughly 100-fold), making it possible to measure analyte concentrations by correlation spectroscopy that are as low as 15 pM. Continued progress along these lines may eventually make it possible to record from single fluorophores that are not otherwise immobilized, concentrated, or compartmentalized.

Six more papers report experimental results using the current generation of single-molecule approaches. For reasons that are doubtless obvious, a good deal of single-molecule research has focused on the genome, and on the molecules involved carrying out

the Central Dogma of replication, transcription, and translation. Three papers discuss revealing studies of the physical properties of chromosomes. The paper by Chien & van Noort reviews the state of the art on single-molecule measurements of eukaryotic chromatin structure, using force spectroscopy to explore the mechanical properties of individual chromatin fibers, which involve hierarchical levels of folding of DNA around nucleosomal cores composed of histone octamers. The exact way that chromatin fibers get compacted (and unfolded) has been the subject of considerable controversy, particularly at the highest-order levels of folding, but recent results using forces applied by optical or magnetic tweezers have now begun to unravel some of the mysteries. This paper supplies an excellent guide to those wishing to bone up on the subject. A related paper by Gurunathan & Levitus reports on the use of fluorescence to study nucleosome dynamics, using a combination of single-molecule FRET and fluorescence correlation spectroscopy. They find evidence consistent with the "site exposure" model for gene expression, where DNA can be transiently unwind from its associated histone core (likely, in a sequence-dependent fashion) to expose nucleic acids to the protein machinery of the Central Dogma. Prokaryotes and even bacteriophages have chromosomes too, of course, but these appear to be much simpler in terms of their folding organization than those of eukaryotes. Nevertheless, mechanical rearrangements of the genome and associated proteins lie at the very heart of gene expression in prokaryotes, as well as eukaryotes. The paper by Wang *et al.* looks at the phenomenon of DNA looping, which is known to regulate gene expression in bacteria (for example, in the *lac* operon), and which can occur when distant regions of DNA are brought together by bound control proteins, such as repressors. In λ bacteriophage, DNA looping is involved in the key decision to become lysogenic. Wang and coworkers studied such looping using a combination of imaging methods, including electron microscopy and atomic force microscopy.

Single-molecule methods can also be used to study protein mobility, or to gauge the strength of receptor-ligand interactions. Winther & Oddershede used an optical trap arrangement to measure the lateral mobility of the λ receptor in *E. coli*, which is found in the outer membrane of cells. By engineering a recombinant version of this receptor to carry a biotinylation site, they were able to attach a streptavidin-coated bead to the extracellular domain of the receptor, and thereby to measure its diffusional freedom via laser light scattered light from the bead, supplied by a low-power optical trap. They used this system to explore the effects of various antibiotics (ampicillin, vancomycin, and two antimicrobial peptides) on cell wall formation and stability, assayed indirectly through the mobility of the λ receptor.

Single molecule spectroscopy has emerged as the experimental tool of choice for investigating protein dynamics, particularly when trying to identify sources of temporal (or spatial) inhomogeneity in structure and function. By studying one molecule at a time, it not only becomes possible to reconstruct the population (bulk) behavior, but also to learn about the population variance, and to study any outlying (or rare) properties of potential interest, including non-Markovian behavior. Furthermore, single molecule spectroscopy can bypass a need for synchronized populations of identical, prepared molecules that is so often a prerequisite for bulk studies. The paper by Lu takes these issues up in the specific context of structural fluctuations in the calcium sensor, calmodulin. Finally, a contribution by Atilgan & Ovryn examines the nucleation of integrin-based adhesion sites on the surfaces of cells. Mature adhesion complexes help cells to adhere to the matrix on the extracellular side of the membrane, and are anchored to cytoskeletal elements on the cytoplasmic face, serving to bridge the cell to its environment. The maturation of "nouveau" adhesions, as these recruit additional proteins and adjust their size and shape, has been a particular matter of interest. This study used a combination of fluorescence microscopy and a technique that the authors refer to as "phase shifting laser feedback interferometry" to measure the tiny distances between the ventral surfaces of cells and their substrata, and thereby to map the membrane surface topography with a precision of several nanometers. Although not a single-molecule study, in the formal sense of the SMB conference, their technique achieves a performance that beats the traditional diffraction limit, and therefore represents one of several novel techniques that are currently pushing the boundaries of light microscopy.

Without doubt, single molecule biophysics has a bright future ahead, and requests have already been received for a reprise of the meeting in 2011. We'll have to see. The job of organizing scientists is often compared to herding cats, which is alternately difficult and frustrating. That said, the results thus far have been immensely gratifying. In closing, I'd like to acknowledge the assistance of the five grad students from my Stanford lab who served as meeting "gophers," fulfilling a myriad of errands: Peter Anthony, Kirsten Frieda, Nick Guydosh, Matt Larson, and Christian Perez, together with the highly professional services of Ms. Jane Kelly, the Administrative Vice President of the ACP, and her able staff, who help to make the ACP such a special place for us scientists to gather.

Steven M. Block
Guest Editor – Current Pharmaceutical Biotechnology
Meeting Organizer, SMB 2009
S.W. Ascherman Professor of Sciences
Departments of Biology and Applied Physics
Stanford University, Stanford, CA 94305
USA