The International Society for Optical Engineering

Nanotechnology E-Newsletter

March 2005

Prompt identification of bacteria from 'fatal screams' caused by phage infection

An interdisciplinary research project at Texas A&M University has discovered that unusual voltage fluctuations will occur in a nanoantenna immersed in a solution containing bacteriophage-infected bacteria. This has lead to a novel technology to detect living bacteria within several minutes, with unparalleled specificity. So far all the existing technologies for bacteria identification require hours to days of culturing or preprocessing. The new technology, named SEPTIC (sensing phage-triggered ion cascade), is potentially a breakthrough in pathogenic bacteria diagnosis.[1]

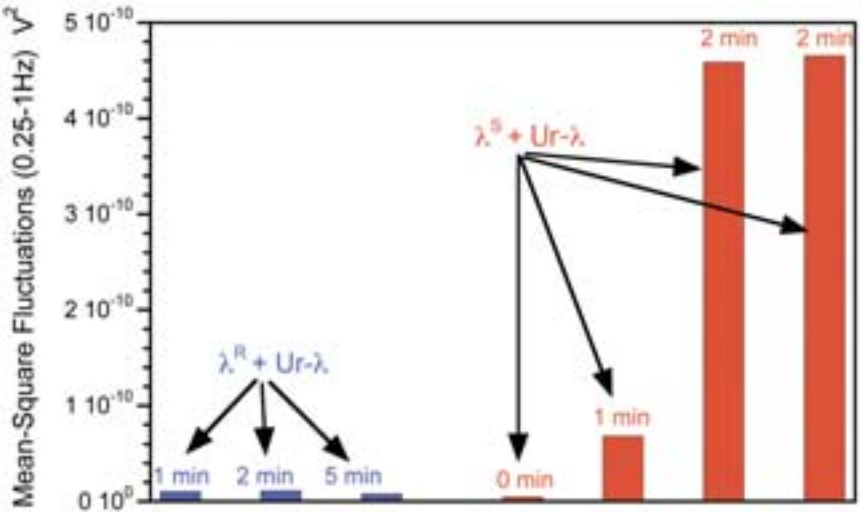

Figure 1. Comparison of the SEPTIC response (red) with the background noise (blue) which is the response to irrelevant phages. The different times indicate different periods of incubation.

Bacteriophage ('phage') is a virus that infects bacteria, and injecting its DNA (deoxyribonucleic acid) into a host. The phage DNA will replicate itself and, after several minutes, the replicated phage-DNA molecules escape, while the host dies. Many tailed bacteriophages cause massive, transitory ion leakages from host cells as a consequence of the phage-DNA injection. This phenomenon is an ideal opportunity for bacterial diagnostics, because it not only takes advantage of the well-known specificity available in the bacteriophage but it can occur within seconds after admixture of the virions and cells. Moreover, it requires no culturing of the analyte but only that the target cells be physiologically viable (i.e., have energized or intact membranes capable of energization).

We constructed a nanowell comprised of two 4μm-wide titanium electrodes separated by a gap of 150nm. A preamplifier and a spectral analyzer were connected to measure the voltage fluctuations of the nanowell when it was immersed in microliter samples containing phage and bacteria. In those mixtures where the bacteria were sensitive to the phage, large stochastic waves with various time and amplitude scales were observed, with power spectra of approximately $1/f^2$ shape in the frequency range of 1-10Hz. In contrast, with mixtures of isogenic-resistant bacteria (where phage infection could not occur), only small 1/f fluctuations due to amplifier noise were observed.

The nanowell acts like an antenna that picks up the nanoscale electric field in the solution. When bacteria are infected by phages and therefore release ions (about 10^8 ions/bacterium), strong noise in the electric field will occur and be captured by the 'antenna'. The unusual noise, whose magnitude and spectrum are completely different than the noise of uninfected bacteria, can be regarded as a 'fatal scream', see Figure 1. Although the kinetics of this are not yet clear, it has been conjectured that it may be caused by the Brownian motion of the released ions as well as the charged bacteria.

When the nano-well antenna is replaced by a microwell, in which the gap between the two 5mm-wide electrodes is 100μm, the fatal screams were no longer heard. The voltage fluctuations in the microwell always followed the 1/f power spectrum even when the bacteria were infected by phages. This indicates that the noise, the fatal scream, was averaged out by the larger size of these antennas.

The tests were conducted with different strains of *Escherichia coli (E. coli)* and different bacteriophages. The experiment showed 100% success rate, i.e., when and only when the *E. coli* strains were sensitive to the phages, fatal screams were observed. This is not surprising, because phages never make mistakes. They never invade the wrong hosts, nor do they miss a target (given high-enough phage concentration).

Detection and identification of bacteria is extremely important. Certain strains of *E. coli* can cause widespread diseases or even death if they get into the food supply chain. Compared to other existing technologies, such as PCR (polymerase chain reaction) or culturing, SEPTIC has significant advantages. First, it is very fast: the fatal screams of bacteria were heard within minutes after introducing phages. Other techniques require hours or even days. Second,

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its false positive or negative probability is extremely small, if not zero. Third, it is based on a small biochip that is suitable for mass production, very cheap, and portable. Other techniques usually involves very bulky and expensive instrumentation.

So far, SEPTIC is the only method suitable for wide deployment in field surveillance. In the future, it will be straightforward to build a biochip with hundreds of nano-antennas, immobilized phages, microfluidic reservoirs and channels, and preamplifiers, to allow the identification of a large variety of bacteria. Based on the current test data, it is estimated that a single bacterium can be identified if the preamplifier is integrated with the nano-antenna on the same chip. The applications of SEPTIC in the life sciences are also promising. For example, a SEPTIC chip can monitor ion transportation related to injuries or the functioning of cells.

A short movie illustrating the working principle of SEPTIC is available at:
http://ee.tamu.edu/~mcheng/SEPTIC

M. Cheng*, M. Dobozi-King, R. Young, and L. B. Kish*
Department of Electrical Engineering
*Department of Biochemistry and Biophysics
Texas A&M University, TX
E-mail: Laszlo.Kish@ee.tamu.edu

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Nanotechnology at Photonics West

Nanotechnology was much in evidence at the recent Photonics West meeting in San Jose, CA. Whole programs were devoted to *Nano/Biophotonics, Laser Micro/Nanoengineering and Applications,* and *Nanotechnologies in Photonics,* and these were supplemented by other free-standing conferences in the same broad area.

On Tuesday 26th January, I chaired a late-evening meeting of the SPIE Technical Group on Nanotechnology at the Fairmont Hotel. This brought together a small but vocal audience drawn from the conference and exhibition attendees. Those present engaged in a lively discussion of personal views concerning the increasingly widespread use of the term 'nanotechnology', and also the extent to which industry is responding to advances such as those being reported in courses and conferences at Photonics West.

It was generally agreed that, with the spread of topics embraced by the term 'nanotechnology', secondary qualifiers of the term are often required to convey a particular subject focus such as quantum dots, molecular motors, and so forth. Indeed some still champion the view that much of nanotechnology is essentially a renaming of what was once called chemistry. It was also agreed that a signal benefit of using the term 'nanotechnology' is that it very effectively brings together those who are involved in what might otherwise seem extremely disparate areas of science and technology: this is proving extremely successful in fostering new trans-disciplinary interactions.

The papers presented at Photonics West will soon be available as SPIE Proceedings. For further information, go to: http://spie.org/Conferences/Programs/05/pw/ and click on 'Proceedings' in the left-hand column.

David L. Andrews
Nanostructures and Photomolecular Systems
School of Chemical Sciences
University of East Anglia
Norwich, UK
E-mail: david.andrews@physics.org
http://www.uea.ac.uk/~c051

Computer-based design of novel protein structures

Proteins are the ultimate nanomachines. They perform sophisticated actions on a smaller scale than any device created by humans. Proteins can enhance chemical reaction rates by over ten orders of magnitude, transfer chemical energy into mechanical force, and can be used to create highly-controllable signaling networks.

Naturally-occurring proteins are the product of thousands of years of evolution and natural selection, but the basis for their activity is grounded in the rules of chemistry and physics. Thus, it is not surprising that for the last 30 years researchers have been trying to learn how to design their own proteins. Unlike many polymers, most functional proteins adopt unique three-dimensional structures. The specific arrangement of key chemical groups allows them to bind other molecules, and in some cases to catalyze chemical reactions. Because it is directly related to function, most research in the area of protein design has focused on the creation of a well-defined structure.

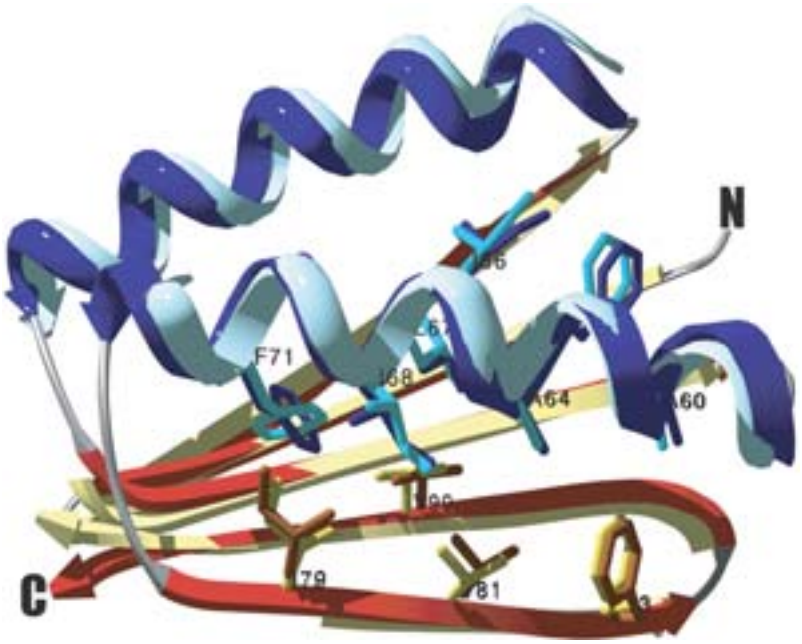

Figure 1. Superposition of the design model of Top7 with its high-resolution crystal structure.

The first efforts in this area were based primarily on heuristics gleaned from studying naturally-occurring proteins. The various amino acids have preferences for the different types of secondary structure, and some interact more favorably with water. Using this approach there were some impressive successes, especially with all-helical proteins. However, in general, the designed proteins did not have well-packed interiors characteristic of those in nature.[1]

In the 1990s, computer algorithms were developed that could help solve this problem.[2] These programs searched for amino acids that could fit together like a jigsaw puzzle when built onto the target-protein structure. These methods were first tested by using them to redesign naturally-occurring protein backbones. Often the designs were quite successful and, in many cases, it was possible to enhance the protein stability. However, although impressive, these results did not really address the long-term goal of protein designers: the creation of new protein structures that can perform novel functions.

Designing a completely-new protein structure is inherently more challenging than redesigning an already existing one because, from the beginning, it is not clear if the target structure is physically possible. In fact, it is anticipated that most protein structures will not be designable. Therefore, to design a new structure it is necessary to search through conformational as well as sequence space. In their landmark design of a four-helix coiled-coil, Harbury and co-workers used an analytical parameterization of packing angles to search a set of coiled-coil backbone conformations.[3] The designed protein matched the design model almost exactly. This study highlighted the need for searching through conformational space, but did not represent an approach that would work for irregular structures.

We recently developed an approach for designing novel protein structures that makes use of the Rosetta program for structure prediction. Starting with a model of the target structure, the design procedure iterates between sequence design and backbone optimization. The sequence design is performed with a Monte Carlo optimization procedure that searches through the preferred rotamers and amino acids at each position. The design procedure is fast (<10 minutes for a 100-residue protein on a single desktop processor) and independent simulations converge to similar sequences and energies.

The backbone optimization also uses a Monte Carlo procedure to sample small changes in backbone dihedral angles. Each perturbation is followed by gradient-based optimization of neighboring dihedral angles before evaluating the move with the Metropolis criterion. A typical simulation involves 10-sequence optimizations, each separated by several thousand backbone-optimization steps. In general, the backbone does not move dramatically during this procedure (<2Å root-mean-square deviation), but the calculated energies and sequence will often change significantly.

To demonstrate the effectiveness of this protocol we designed a protein with a topology that is not found in the *Protein Data Bank* (see Figure 1).[4] The protein, called Top7, contains five *b*-strands and two *a*-helices. Top7 is exceptionally stable and remains folded up to 100°C. Further, the high-resolution crystal structure of Top7 is nearly identical to the design model. This is the first time that any laboratory has used a general approach to accurately design a new protein structure, and is an important stepping stone towards the rational design of novel protein nanomachines. We anticipate that the coupling of sequence-design algorithms with structure prediction methodology will be a powerful approach for designing new protein-protein interactions and new protein-active sites.

Brian Kuhlman, Gautam Dantas*, and David Baker*
Department of Biochemistry and Biophysics
University of North Carolina, Chapel Hill
E-mail: bkuhlman@email.unc.edu
*Department of Biochemistry
University of Washington
Seattle, WA

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Optical properties of nanoparticles: size, interface and substrate effects

The optical properties of metallic nanoparticles have been extensively studied for a long time,[1-7] but recent advances in nanotechnology have brought this fascinating area to the forefront of current scientific research. As a result, a new area of subdiffractional optics has appeared: known as plasmonics, it applies where light is supposed to play the role of information carrier rather than the electrons used in traditional micro- and nanoelectronics. Metal and semiconductor nanoparticles—arranged either in space or on a substrate—may be used for the development of a new class of plasmonic devices for integrated nanophotonic circuits.

Nanoparticles, when deposited on a dielectric or semiconductor substrate and irradiated with an external light beam, induce surface charges at the interface between the substrate and ambient medium. As a result, the local electric field acting on the nanoparticles is modified and the resonance extinction of the light is red-shifted with respect to the single nanoparticle spectrum.

Figure 1 depicts the measured and calculated optical extinction of 2nm silver nanoparticles deposited on a semiconductor substrate. The theoretical extinction has been calculated using the electrostatic approximation—valid for nanoparticles with sizes much smaller than the wavelength of the incident light—taking into account the induced charges at the interface between the substrate and the ambient medium.[3] A so-called A-parameter was used to modify the dielectric function of the silver nanoparticles in order to take into account the size effect. This is caused by the damping of the plasmon excitation due to the scattering of free electrons from the surface of the nanoparticles.

In order to describe the optical response of nanoparticles, one needs the frequency dependence of the dielectric function of the particle's constituent material. The Drude model for the dielectric function, which describes the behavior of free conduction electrons, can be used for noble metallic nanoparticles. In the visible range, however, interband electronic transitions play an important role in the optical response: these should be taken into account to get the correct description of the resonance frequency and bandwidth of the plasmon excitation.

We developed a simple method for determining the resonance frequency and bandwidth of the plasmon excitation in noble metallic nanoparticles, taking into account the interband electronic transitions in the dielectric function.[4] Figure 2 depicts the proposed idea, where the resonance response of a 5nm silver nanoparticle is calculated using the electrostatic approximation together with the decomposed complex dielectric function of the bulk silver.

Silver nanoparticles embedded in different host media demonstrate a broadened resonance linewidth as compared with theoretical calculations.[5,6] To properly describe the broadening effect, we developed a so-called interface-decay channel theory, which enables us to describe the plasmon damping caused by the presence of adsorbed molecules on the surface of the particle.[5,6] A new approach has also been proposed for determining the interface electronic characteristics (i.e. projected density of

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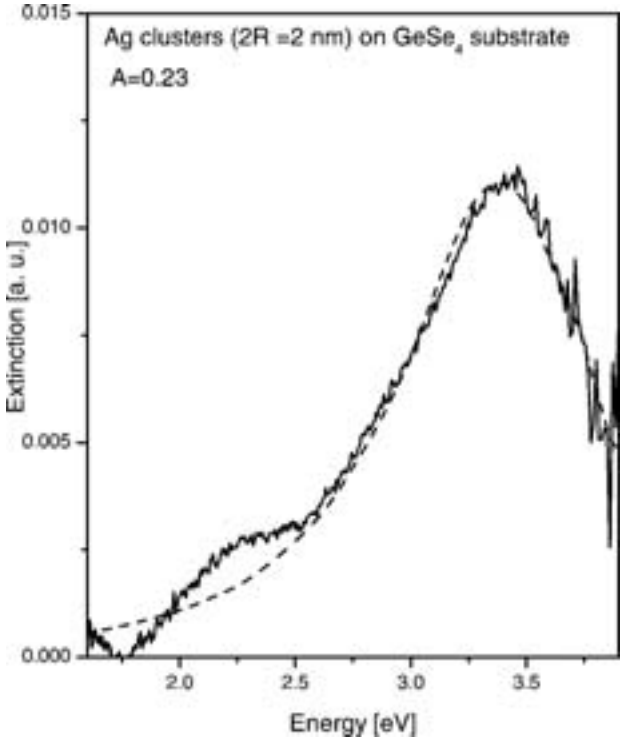

Figure 1. Extinction cross-section of 2nm silver nanoparticles deposited on a semiconductor substrate as a function of the incident photon energy.

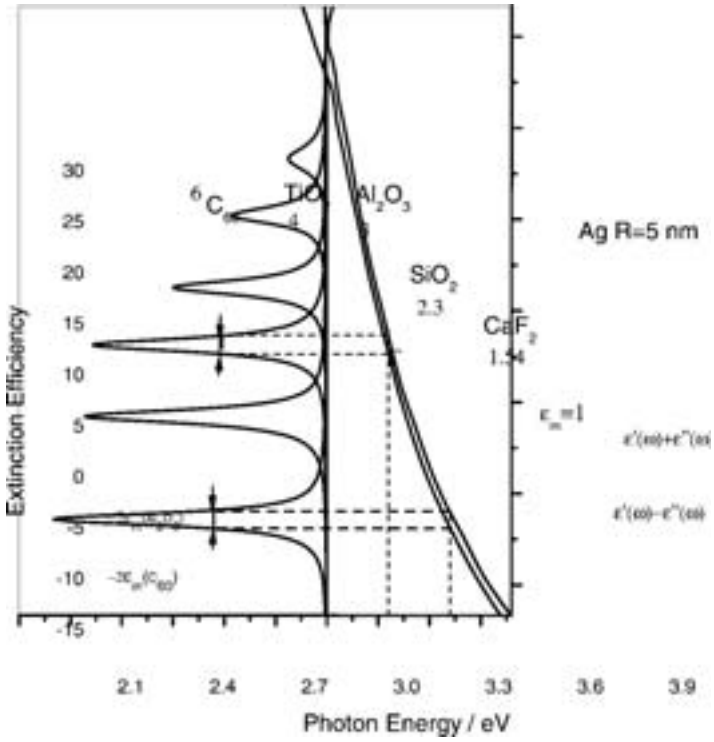

Figure 2 . Calculated extinction cross section of a 5nm silver nanoparticle embedded in different host materials, as a function of the photon energy and the dielectric function of bulk silver.

Optical properties of nanoparticles: size, interface and substrate effects

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states of adsorbed molecules) based on the optical experiments.[5]

An interesting phenomenon of optical bistability in semiconductor nanoparticles has emerged, which may be used for the development of new nanophotonic switching devices. To aid in this process, we have developed an effective-medium theory for the calculation of the effective dielectric function of a suspension of semiconductor ellipsoidal nanoparticles.[7]

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Anatoliy Pinchuk
Institute of Surface Chemistry
General Naumov Str. 17
03164 Kiev, Ukraine
E-mail: pinchuk@physik.rwth-aachen.de

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Direct imaging of transcription repressors bound to nano-channel elongated DNA

This note reports the direct imaging of the *E.-coli-lac* repressor protein (LacI) bound to DNA (deoxyribonucleic acid): in this case, DNA with a single copy of the *Lac* operator (*lacO*) inserted in it (40.82kbp). One of the key challenges in transcription profiling is the observation of interactions of transcription-factor proteins with single DNA molecules. Current technology, such as DNA footprinting analysis, looks at an ensemble of millions of DNA strands. In order to analyze the occupancy of transcription factor sites by individually-bound proteins, it is necessary that the DNA molecule be extended in a linear manner and that single proteins be imaged with high spatial resolution. We have developed a platform using single-molecule fluorescence imaging and nano-fabrication techniques to directly visualize transcription factor proteins on DNA to a resolution of 100 base pairs (bp) .

In order to visualize the LacI, a protein was constructed that fused green fluoresecent protein (GFP) and LacI. The monomeric GFP-LacI fusion proteins were bound *in vitro* to *lacO*-DNA constructs. The DNA molecules were then stained with the red fluorescent intercalator dye called BOBO-3, and the LacI-DNA complex molecules elongated in nano-channels with dimensions comparable to the persistence length of double-stranded DNA (60nm). Next, the DNA and protein were imaged using total-internal-reflection fluorescence (TIRF) microscopy.

Figure 1(a) shows the micro-/nano-fluidic device. The idea was to drive the DNA molecules into the micro-channel, and then into the nano-channels, using electrophoresis. Figure 1(b) shows GFP-LacI bound to a *lacO*-DNA construct elongated in a nano-channel. The location of the bound protein was determined by curve-fitting the DNA fluorescence-intensity profile to a modified error function,[1,2] and the protein profile to a Gaussian function.[2] The precision of the protein location is set by the measurement error associated with the positions of the two DNA ends (i.e. DNA length) and the position of the protein in the image. The error in DNA-length measurements decreases with N (the number of independent measurements[1]) as $1/N^{1/2}$. The error in protein location measurements, on the other hand, decreases with M (the number of collected photons from a GFP-LacI molecule[3]) as $1/M^{1/2}$.

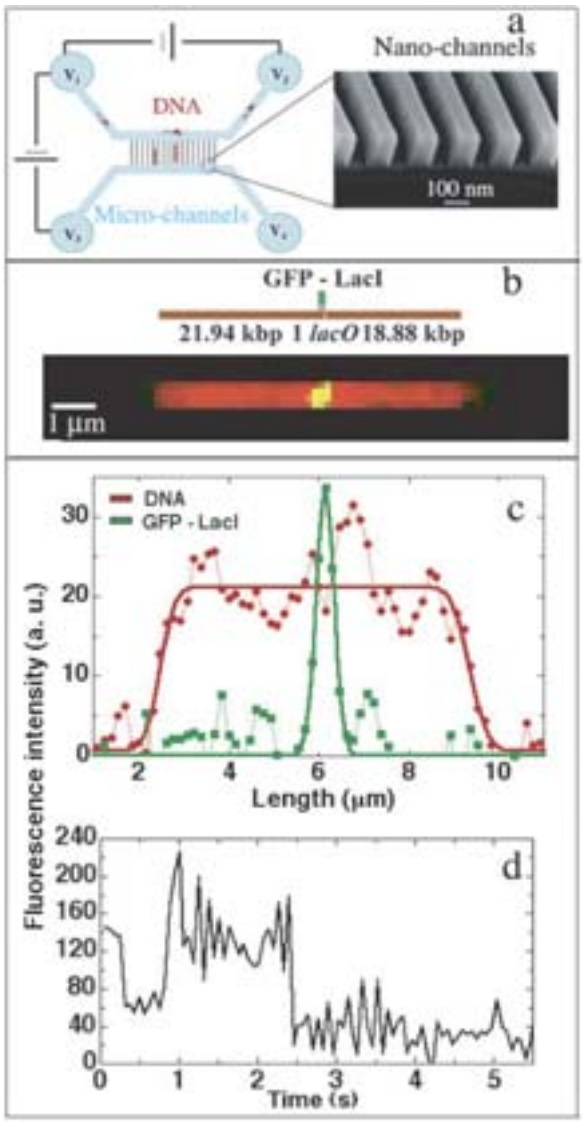

Figure 1. Micro- and nano-fluidic device (unsealed), and image and analysis of GFP-LacI bound to lacO*-DNA. (a) Schematics of the micro-/nano-fluidic device. Blue regions are micro-channels and the bridging black lines are nano-channels along which the DNA molecules (red) are elongated. DNA molecules are guided consecutively into micro- and nano-channels using electrophoresis. Scanning electron microscopy images of an array of 80nm×100nm channels made using nano-imprint lithography.[4] (b) Schematics of GFP-LacI bound to* lacO*-DNA, and (superposed) a frame-averaged image of LacI-DNA elongated in a 100nm×100nm channel. (c) Fluorescence intensity profiles and fits for the DNA and the GFP-LacI. (d) Fluorescence time trace of the bound GFP-LacI, which emitted 6.3×10^4 photons in its lifetime.*

The DNA molecule shown in Fig. 1(b) is an averaged image of 150 frames and the bound GFP-LacI is an averaged image of 40 frames. This gives an average M of 34 photons per frame. The protein is located 170bp off the target *lacO* site, and it is within the measurement error of approximately 190bp. By taking hundreds more images of the DNA, the bound location can be determined to the precision of 100bp, or limited by the number of photons had the GFP-LacI molecules a higher photon yield.[2] This GFP-LacI molecule was bound specifically to *lacO*: it is a monomer according to the photon yield of 6.3×10^4 photons, and the unitary bleaching event observed in the fluorescence time trace.[2]

Y. M. Wang, Keith Morton*, Xiao-Juan Guan*, Ling Guo*, Edward C. Cox*, James Sturm†, Steve Chou[3], Shirley Tilghman*, and Robert H. Austin
Department of Physics
*Department of Molecular Biology
†Department of Electrical Engineering
Princeton University
Princeton, NJ
E-mail: ymwang@Princeton.EDU

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Tel: +1 360 676 3290. Fax: +1 360 647 1445.

European Office: Karin Burger, Manager, karin@spieeurope.org, Tel: +44 7974 214542. Fax: +44 29 2040 4873.

In Russia/FSU: 12, Mokhovaja str., 119019, Moscow, Russia • Tel/Fax: +7 095 202 1079
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