

Douglas E. Smith – Abstracts of Research Papers written at UCSD, Grouped by Subject

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1. Viral DNA Packaging Forces and Molecular Motors (in Bacteriophages ϕ 29, λ , and T4)

Single bacteriophage T4 DNA packaging motors exhibit large force generation, high velocity, and dynamic variability

D.N. Fuller, D.M. Raymer, V. Kottadiel, V.B. Rao, and D.E. Smith,

Proceedings of the National Academy of Sciences USA 104, 16868 (2007)

Terminase enzyme complexes, which facilitate ATP-driven DNA packaging in phages and many eukaryotic viruses, constitute a wide and potentially diverse family of molecular motors about which little dynamic-mechanistic information is available. Here, we report optical tweezers measurements of single DNA molecule packaging dynamics in phage T4, a large tailed *E. coli* virus that is an important model system in molecular biology. We show that a complex is formed between the empty prohead and the large terminase protein (gp17) that can capture and begin packaging a target DNA molecule within a few seconds, thus demonstrating a distinct viral assembly pathway. The motor generates forces >60 piconewtons (pN), similar to those measured with ϕ 29, suggesting that high force generation is a common property of viral DNA packaging motors. However, the DNA translocation rate was strikingly higher than that of ϕ 29, averaging ~700 bp/s and ranging up to ~2000 bp/s, consistent with packaging by phage T4 of an enormous 171 kb genome in <10 minutes during viral infection, and implying high ATP turnover rates of >300 s⁻¹. The motor velocity decreased with applied load, but averaged 320 bp/s at 45 pN, indicating very high power generation. Interestingly, the motor also exhibited large dynamic changes in velocity, suggesting that it can assume multiple active conformational states gearing different translocation rates. This capability, in addition to reversible pausing and slipping capabilities that were observed, may allow phage T4 to coordinate DNA packaging with other ongoing processes, including viral DNA transcription, recombination, and repair.

Measurements of single DNA molecule packaging dynamics in bacteriophage λ reveal high forces, high motor processivity, and capsid transformations

D.N. Fuller, D. Raymer, J.P. Rickgauer, R.M. Robertson, C.E. Catalano, D.L. Anderson, S. Grimes, D.E. Smith,

Journal of Molecular Biology 373, 1113 (2007)

Molecular motors drive genome packaging into preformed procapsids in many dsDNA viruses. Here, we present optical tweezers measurements of single DNA molecule packaging in bacteriophage λ . DNA-gpA-gpNu1 complexes were assembled with recombinant gpA and gpNu1 proteins and tethered to microspheres, and procapsids were attached to separate microspheres. DNA binding and initiation of packaging were observed within a few seconds of bringing these microspheres into proximity in the presence of ATP. The motor was observed to generate greater than 50 piconewtons (pN) of force, in the same range as observed with bacteriophage ϕ 29, suggesting that high force generation is a common property of viral packaging motors. However, at low capsid filling the packaging rate averaged ~600 bp/s, which is 3.5-fold higher than ϕ 29, and the motor processivity was also 3-fold higher, with less than one slip per genome length translocated. The packaging rate slowed significantly with increasing capsid filling, indicating a buildup of internal force reaching 14 pN at 86% packaging, in good agreement with the force driving DNA ejection measured in osmotic pressure experiments and calculated theoretically. Taken together, these experiments show that the internal force that builds during packaging is largely available to drive subsequent DNA ejection. In addition, we observed an 80 bp/s dip in the average packaging rate at 30% packaging, suggesting that procapsid expansion occurs at this point following the buildup of an average of 4 pN of internal force. In experiments with a DNA construct longer than the wild-type genome, a sudden acceleration in packaging rate was observed above 90% packaging in many cases, and greater than 100% of the genome length was translocated, suggesting that internal force can rupture the immature procapsid.

Initiation of bacteriophage ϕ 29 DNA packaging and effect of the gp3 terminal protein studied by an optical tweezers assay.

J.P. Rickgauer, D.N. Fuller, S. Grimes, P.J. Jardine, D.L. Anderson, D.E. Smith, submitted

A key step in the assembly of many viruses is the packaging of DNA into a precursor capsid (prohead) by the action of an ATP-powered molecular motor. Here we describe a new optical tweezers assay that permits study of the initiation of DNA packaging into single bacteriophage ϕ 29 proheads. We demonstrate an assembly sequence whereby prohead-ATPase complexes are assembled *in vitro* and then bind a target DNA molecule within a few seconds and began translocation within one second. This measurement was facilitated by a microfluidic system that permits rapid loading of two microspheres into two optical traps. We found that the DNA terminal protein gp3 had a dramatic effect on the DNA-gp3 conformation during packaging initiation. The initial extended length of the DNA-gp3 tether varied from ~30-100% of the full-length substrate, showing that packaging did not generally involve binding of a free end of the DNA, and that DNA-gp3 had a higher-order structure. Digestion of gp3 with proteinase K eliminated this variability, resulting in uniform full-length tethers at initiation. These findings are consistent with previous electron microscopy studies showing that ϕ 29 DNA-gp3 forms lariats mediated by gp3 and suggest that DNA packaging initiates at the lariat loop junctions. In addition, sucrose gradient sedimentation

studies showed that the gp16 motor protein could cleave the gp3-DNA, providing an explanation for the translocation of the short tethers in the optical tweezers assay. We found that this effect could also be avoided by using a DNA substrate lacking gp3. In particular, non-native DNAs generated by PCR, including *E. coli* and human sequences, were readily packaged with reproducible DNA terminal initiation, facilitating accurate measurement of the length of DNA packaged.

Ionic effects on viral DNA packaging and portal motor function in bacteriophage ϕ 29

D.N. Fuller, J.P. Rickgauer, P.J. Jardine, S. Grimes, D.L. Anderson, D.E. Smith

Proceedings of the National Academy of Sciences USA 104, 11245 (2007).

In many viruses, DNA is confined at such high density that its bending rigidity and electrostatic self-repulsion present a strong energy barrier in viral assembly. Therefore, a powerful molecular motor is needed to package the DNA into the viral capsid. Here, we investigate the role of electrostatic repulsion on single DNA packaging dynamics in bacteriophage ϕ 29 via optical tweezers measurements. We show that ionic screening strongly affects the packing forces, confirming the importance of electrostatic repulsion. Separately, we find that ions affect the motor function. We separate these effects through constant force measurements and velocity versus load measurements at both low and high capsid filling. Regarding motor function, we find that eliminating free Mg^{2+} blocks initiation of packaging. In contrast, Na^+ is not required, but it increases the motor velocity by up to 50% at low load. Regarding internal resistance, we find that the internal force was lowest when Mg^{2+} was the dominant ion or with the addition of 1 mM Co^{3+} . Forces resisting DNA confinement were up to ~80% higher with Na^+ as the dominant counterion and only ~90% of the genome length could be packaged in this condition. The observed trend of the packing forces is in accord with that predicted by DNA charge screening theory. However, the forces are up to six times higher than predicted by models that assume coaxial spooling of the DNA and interaction potentials derived from DNA condensation experiments. The forces are also several-fold higher than ejection forces measured with bacteriophage λ .

Portal motor velocity and internal force resisting viral DNA packaging in bacteriophage ϕ 29

J.P. Rickgauer, D. Fuller, S. Grimes, P.J. Jardine, D.L. Anderson, D.E. Smith

Biophysical Journal 94, 159 (2008)

During the assembly of many viruses, a powerful molecular motor compacts the genome into a pre-assembled capsid. Here, we present measurements of viral DNA packaging in bacteriophage ϕ 29 using an improved optical tweezers method that allows DNA translocation to be measured from initiation to completion. This method allowed us to study the previously uncharacterized early stages of packaging and facilitated more accurate measurement of the length of DNA packaged. We measured the motor velocity versus load at near-zero filling and developed a ramped DNA stretching technique that allowed us to measure the velocity versus capsid filling at near-zero load. These measurements reveal that the motor can generate significantly higher velocities and forces than detected previously. Towards the end of packaging the internal force resisting DNA confinement rises steeply, consistent with the trend predicted by many theoretical models. However, the force rises to a higher magnitude, particularly during the early stages of packaging, than predicted by models that assume coaxial inverse spooling of the DNA. This finding suggests that the DNA is not arranged in that conformation during the early stages of packaging and indicates that internal force is available to drive complete genome ejection *in vitro*. The maximum force exceeds 100 piconewtons, which is about one-half that predicted to rupture the capsid shell.

2. Protein-induced DNA looping and cleavage

Tension-dependent DNA cleavage by restriction endonucleases: two-site enzymes are "switched off" at low force

G.J. Gemmen, R. Millin, D.E. Smith

Proceedings of the National Academy of Sciences USA 103, 11555 (2006)

DNA looping occurs in many important protein-DNA interactions, including those regulating replication, transcription, and recombination. Recent theoretical studies predict that tension of only a few piconewtons acting on DNA would almost completely inhibit DNA looping. Here, we study restriction endonucleases that require interaction at two separated sites for efficient cleavage. Using optical tweezers we measured the dependence of cleavage activity on DNA tension with 15 known or suspected two-site enzymes (BfiI, BpmI, BsgI, BspMI, Cfr9I, Cfr10I, Eco57I, EcoRII, FokI, HpaII, MboII, NarI, SacII, Sau3AI, and SgrAI) and six one-site enzymes (BamHI, EcoRI, EcoRV, HaeIII, HindIII, and DNaseI). All of the one-site enzymes were virtually unaffected by 5 pN of tension, whereas all of the two-site enzymes were completely inhibited. These enzymes thus constitute a remarkable example of a tension sensing "molecular switch." A detailed study of one enzyme, Sau3AI, indicated that the activity decreased exponentially with tension and the decrease was ~10-fold at 0.7 pN. At higher

forces (~20–40 pN) cleavage by the one-site enzymes EcoRV and HaeIII was partly inhibited and cleavage by HindIII was enhanced, whereas BamHI, EcoRI, and DNaseI were largely unaffected. These findings correlate with structural data showing that EcoRV bends DNA sharply, whereas BamHI, EcoRI, and DNaseI do not. Thus, DNA-directed enzyme activity involving either DNA looping or bending can be modulated by tension, a mechanism that could facilitate mechanosensory transduction *in vivo*.

DNA looping by two-site restriction endonucleases: heterogeneous probability distributions for loop size and unbinding force. *G.J. Gemmen, R. Millin and D.E. Smith*

Nucleic Acids Research 34, 2864 (2006)

Proteins interacting at multiple sites on DNA via looping play an important role in many fundamental biochemical processes. Restriction endonucleases that must bind at two recognition sites for efficient activity are a useful model system for studying such interactions. Here we used single DNA manipulation to study sixteen known or suspected two-site endonucleases. In eleven cases (BpmI, BsgI, BspMI, Cfr10I, Eco57I, EcoRII, FokI, HpaII, NarI, Sau3AI and SgrAI) we found that substitution of Ca²⁺ for Mg²⁺ blocked cleavage and enabled us to observe stable DNA looping. Forced disruption of these loops allowed us to measure the frequency of looping and probability distributions for loop size and unbinding force for each enzyme. In four cases we observed bimodal unbinding force distributions, indicating conformational heterogeneity and/or complex binding energy landscapes. Measured unlooping events ranged in size from 7 to 7500 bp and the most probable size ranged from less than 75 bp to nearly 500 bp, depending on the enzyme. In most cases the size distributions were in much closer agreement with theoretical models that postulate sharp DNA kinking than with classical models of DNA elasticity. Our findings indicate that DNA looping is highly variable depending on the specific protein and does not depend solely on the mechanical properties of DNA.

Dynamics of single DNA looping and cleavage by Sau3AI and effect of tension applied to the DNA

G.J. Gemmen, R. Millin, D.E. Smith

Biophysical Journal 91, 4154 (2006)

Looping and cleavage of single DNA molecules by the two-site restriction endonuclease Sau3AI were measured with optical tweezers. A DNA template containing many recognition sites was used, permitting loop sizes from ~10 to 10,000 basepairs. At high enzyme concentration cleavage events were detected within 5 seconds and nearly all molecules were cleaved within 5 minutes. Activity decreased ~10-fold as the DNA tension was increased from 0.03 to 0.7 pN. Substituting Ca²⁺ for Mg²⁺ blocked cleavage, permitting measurement of stable loops. At low tension, the initial rates of cleavage and looping were similar (~0.025 s⁻¹ at 0.1 pN), suggesting that looping is rate limiting. Short loops formed more rapidly than long loops. The optimum size decreased from ~250 to 45 bp and the average number of loops (in 1 minute) from 4.2 to 0.75 as tension was increased from 0.03 to 0.7 pN. No looping was detected at 5 pN. These findings are in qualitative agreement with recent theoretical predictions considering only DNA mechanics, but we observed weaker suppression with tension and smaller loop sizes. Our results suggest that the span and elasticity of the protein complex, nesting of loops, and protein-induced DNA bending and wrapping play an important role.

3. Nucleosome Unraveling

Forced Unraveling of Nucleosomes Assembled on Heterogeneous DNA Using Core Histones, NAP-1, and ACF

G.J. Gemmen, R. Sim, K.A. Haushalter, P.C. Ke, J.T. Kadonaga, D.E. Smith

Journal of Molecular Biology 351, 89 (2005)

Periodic arrays of nucleosomes were assembled on heterogeneous DNA using core histones, the histone chaperone NAP-1, and ATP-dependent chromatin assembly and remodeling factor (ACF). The mechanical properties of these complexes were interrogated by stretching them with optical tweezers. Abrupt events releasing ~55 to 95 base pairs of DNA, attributable to the non-equilibrium unraveling of individual nucleosomes, were frequently observed. This finding is comparable with a previous observation of 72 - 80 bp unraveling events for nucleosomes assembled by salt dialysis on a repeating sea urchin 5S RNA positioning element, but the unraveling force varied over a wider range (~5 to 65 pN, with the majority of events at lower force). Because ACF assembles nucleosomes uniformly on heterogeneous DNA sequences, as in native chromatin, we attribute this variation to a dependence of the unraveling force on the DNA sequence within individual nucleosomes. The mean force increased from 24 to 31 pN as NaCl was decreased from 100 to 5 mM. Spontaneous DNA re-wrapping events were occasionally observed in real time during force relaxation. The observed wide variations in the dynamic force needed to unravel individual nucleosomes and the occurrences of sudden DNA re-wrapping events may have an important regulatory influence on DNA-directed nuclear processes, such as the binding of transcription factors and the movement of polymerase complexes on chromatin.

4. Single Polymer Dynamics (single DNA molecule dynamics)

Direct measurement of the intermolecular forces confining a single molecule in an entangled polymer solution

R.M. Robertson and D.E. Smith

Physical Review Letters 99, 126001 (2007)

We use optical tweezers to directly measure the intermolecular forces acting on a single polymer imposed by surrounding entangled polymers (115 kbp DNA, 1 mg/ml). A tube-like confining field was measured in accord with the key assumption of reptation models. A time-dependent harmonic potential opposed transverse displacement, in accord with recent simulation findings. A tube radius of 0.8 μm was determined, close to the predicted value (0.5 μm). Three relaxation modes (~ 0.4 , 5 and 34 s) were measured following transverse displacement, consistent with predicted relaxation mechanisms.

Direct measurement of the confining forces imposed on a single molecule in a concentrated solution of circular polymers.

R.M. Robertson and D.E. Smith

Macromolecules 40, 8737 (2007)

We measure the forces confining the displacement of a single DNA molecule embedded within a concentrated solution of long relaxed circular DNA molecules (115 kbp at 1 mg/ml) using optical tweezers. We compare these measurements with our previous measurements of forces imposed by entangled linear DNA molecules of the same length and concentration. A tube-like confining field and three relaxation modes were observed, but the tube radius was 25% lower ($\cong 0.6 \mu\text{m}$) and the longest relaxation time $\sim 3\times$ lower ($\cong 11$ s) than with linear molecules, consistent with recent theoretical predictions by Iyer, Lele, and Juvekar. For displacements greater than the tube radius, the confining force imposed by circular polymers was substantially lower and shorter range than that measured with linear polymers.

Self-diffusion of entangled linear and circular DNA molecules: Dependence on length and concentration

R.M. Robertson and D.E. Smith

Macromolecules 40, 3373 (2007)

Self-diffusion coefficients (D) of DNA molecules of varying length and concentration were measured by tracking the Brownian motion of individual fluorescently labeled tracer molecules. Four possible cases were examined: linear tracer molecules surrounded by linear molecules ($L-L$), circular tracers surrounded by linears ($C-L$), linear tracers surrounded by circles ($L-C$), and circles surrounded by circles ($C-C$). With 6 and 11 kilobasepair DNA D was largely insensitive to topology and varied consistent with Rouse scaling ($D \sim L^{-1}C^{-0.5}$). In contrast, with 25 and 45 kilobasepair DNA topology had a strong influence. At 1 mg/ml we found $D_{C-C} > D_{L-C} \gg D_{L-L} \gg D_{C-L}$. In the $L-L$, $L-C$, and $C-C$ cases a crossover from scaling consistent with the Rouse model to scaling consistent with the reptation model ($D \sim L^{-2}C^{-1.75}$) was observed at $\sim 6\times$ the molecular overlap concentration. In contrast, D_{C-L} decreased much more steeply with concentration, indicating that a process much slower than reptation governs that case.

Strong effects of molecular topology on diffusion of entangled DNA molecules

R.M. Robertson and D.E. Smith

Proceedings of the National Academy of Sciences USA 104, 4824 (2007)

When long polymers such as DNA are in a highly concentrated state they may become entangled, leading to restricted self-diffusion. Here we investigate the effect of molecular topology on diffusion in concentrated DNA solutions and find surprisingly large effects, even with molecules of modest length and concentration. We measured the diffusion coefficients of linear and relaxed circular molecules by tracking the Brownian motion of single molecules with fluorescence microscopy. Four possible cases were compared: linear molecules surrounded by linear molecules, circular molecules surrounded by linear molecules, linear molecules surrounded by circles, and circles surrounded by circles. In measurements with 45 kilobasepair DNA at 1 mg/ml, we found that circles diffused $\sim 100\times$ slower when surrounded by linear molecules than when surrounded by circles. In contrast, linear and circular molecules diffused at nearly the same rate when surrounded by circles, and circles diffused $\sim 10\times$ slower than linears when surrounded by linears. Thus, diffusion in entangled DNA solutions is strongly dependent on topology of both the diffusing molecule and the surrounding molecules. This effect is also strongly dependent on DNA concentration and length. The differences largely disappeared when the concentration was lowered to 0.1 mg/ml or when the DNA length was lowered to 6 kilobases. Present theories cannot fully explain these effects.

Diffusion of isolated DNA molecules: Dependence on length and topology

R.M. Robertson, S. Laib, D.E. Smith

Proceedings of the National Academy of Sciences USA, 103, 7310 (2006)

The conformation and dynamics of circular polymers is a subject of considerable theoretical and experimental interest. DNA is an important example because it occurs naturally in different topological states, including linear, relaxed circular, and supercoiled circular forms. A fundamental question is how the diffusion coefficients of isolated polymers scale with molecular length and how they vary for different topologies. Here, diffusion coefficients D for relaxed circular, supercoiled,

and linear DNA molecules of length L ranging from ~ 6 to 290 kbp were measured by tracking the Brownian motion of single molecules. A topology-independent scaling law $D \sim L^\nu$ was observed with $\nu_L = 0.571 \pm 0.014$, $\nu_C = 0.589 \pm 0.018$, and $\nu_S = 0.571 \pm 0.057$ for linear, relaxed circular, and supercoiled DNA, respectively, in good agreement with the scaling exponent of $\nu = 0.588$ predicted by renormalization group theory for polymers with significant excluded volume interactions. Our findings thus provide evidence in support of several theories that predict an effective diameter of DNA much greater than the Debye screening length. In addition, the measured ratio $D_{\text{Circular}}/D_{\text{Linear}} = 1.32 \pm 0.014$ was closer to the value of 1.45 predicted by using renormalization group theory than the value of 1.18 predicted by classical Kirkwood hydrodynamic theory and agreed well with a value of 1.31 predicted when incorporating a recently proposed expression for the radius of gyration of circular polymers into the Zimm model.

Preparation and Characterization of a Set of Linear DNA Molecules for Polymer Physics and Rheology Studies

S. Laib, R.M. Robertson, and D.E. Smith

Macromolecules, 39, 4115 (2006)

Imaging of single DNA molecules has enabled detailed studies of dilute polymer dynamics and rigorous testing of assumptions and predictions of molecular theories. It is of interest to extend these methods to the study of entangled polymers and to correlate molecular dynamics with rheology measurements. Progress in this direction has been hampered, however, by a lack of available DNA samples in sufficient quantities and covering a wide range of lengths. Here we describe the preparation of a suitable set of molecules ranging in length from ~ 3 to 300 kilobasepairs. These constructs are replicated as plasmids or as fosmids or bacterial artificial chromosomes fitted with an inducible high-copy number origin of replication. DNA sequences were chosen to allow molecules to be linearized by single-cutting restriction enzymes. We show that these molecules can be imaged by fluorescence microscopy and can be prepared in sufficient quantities for bulk rheology measurements.

5. Physical knot formation

Spontaneous knotting of an agitated string

D.M. Raymer and D.E. Smith,

Proceedings of the National Academy of Sciences USA 104, 16432 (2007) (Undergraduate project)

It is well known that a jostled string tends to become knotted; yet the factors governing the “spontaneous” formation of different types of knots are unclear. We performed experiments in which a string was tumbled inside a box and found that complex knots often form within seconds. We used mathematical knot theory to analyze the knots. Above a critical string length, the probability P of knotting at first increased sharply with length, but then saturated below 100%. This behavior differs from that of mathematical self-avoiding random walks, where P has been proven to approach 100%. Finite agitation time and jamming of the string due to its stiffness result in lower probability, but P approaches 100% with long, flexible strings. We analyzed the knots by calculating their Jones polynomials via computer analysis of digital photos of the string. Remarkably, almost all were identified as prime knots: 120 different types, having minimum crossing numbers up to 11, were observed in 3415 trials. All prime knots with up to 7 crossings were observed. The relative probability of forming a knot decreased exponentially with minimum crossing number and Möbius energy, mathematical measures of knot complexity. Based on the observation that a long stiff string tends to form a coiled structure when confined, we propose a simple model to describe the knot formation based on random “braid moves” of the string end. Our model can qualitatively account for the observed distribution of knots and dependence on agitation time and string length.

6. DNA manipulation, Optical tweezers, Methods

A general method for manipulating DNA sequences from any organism with optical tweezers

D.N. Fuller, G.J. Gemmen, J.P. Rickgauer, A. Dupont, R. Millin, P. Recouvreux, D.E. Smith

Nucleic Acids Research 34, e15 (2006)

Mechanical manipulation of single DNA molecules can provide novel information about DNA properties and protein-DNA interactions. Here we describe and characterize a useful method for manipulating desired DNA sequences from any organism with optical tweezers. Molecules are produced from either genomic or cloned DNA by PCR using labeled primers and are tethered between two optically trapped microspheres. We demonstrate that human, insect, plant, bacterial, and viral sequences ranging from ~ 10 to 40 kbp can be manipulated. Force-extension measurements show that these constructs exhibit uniform elastic properties in accord with the expected contour lengths for the targeted sequences. Detailed protocols for preparing and manipulating these molecules are presented, and tethering efficiency is characterized as a function of DNA concentration, ionic strength, and pH. Attachment strength is characterized by measuring the unbinding time as a function of applied force. An alternative stronger attachment method using an amino-carboxyl linkage, which allows for reliable DNA overstretching, is also described.

A simple system for staining protein and nucleic acid electrophoresis gels.

D.M. Raymer, D.E. Smith

Electrophoresis 28, 746 (2007) (Undergraduate project)

Researchers in molecular biology spend a significant amount of time tending to the staining and destaining of electrophoresis gels. Here we describe a simple system, costing ~\$100 and taking ~1 hour to assemble, that automates standard nucleic acid and protein gel staining protocols. Staining is done in a tray or, with DNA gels, in the electrophoresis chamber itself following automatic detection of the voltage drop. Miniature pumps controlled by a microcontroller chip exchange the necessary solutions at programmed time intervals. We demonstrate efficient and highly reproducible ethidium bromide and methylene blue staining of DNA in agarose gels and coomassie blue and silver staining of proteins in polyacrylamide gels.

DNA as a metrology standard for length and force measurements with optical tweezers

J.P. Rickgauer, D.N. Fuller, D.E. Smith

Biophysical Journal 91, 4253 (2006)

Optical tweezers have broad applications in studies of structures and processes in molecular and cellular biophysics. Use of optical tweezers for quantitative molecular-scale measurement requires careful calibration in physical units. Here we show that DNA molecules may be used as metrology standards for force and length measurements. Analysis of DNA molecules of two specific lengths allows simultaneous determination of all essential measurement parameters. We validate this "biological calibration" method experimentally and with simulated data, and show that precisions in determining length scale factor (~0.2%), length offset (~0.03%), force scale factor (~2%), and compliance of the traps (~3%) are limited only by current measurement variation, much of which arises from polydispersity of the microspheres (~2%). We find this procedure to be simpler and more convenient than previous methods, and suggest that it provides an easily replicated standard that can insure uniformity of measurements made in different laboratories.