reaction coordinate, but as a second dimension the distance between ions needs to be taken into account as ions do not move independently through the pore.

The biasing potential in both equilibrium and nonequilibrium methods can be chosen arbitrarily, rendering the PMF approach suitable also for high barriers that are precluded from direct flux estimates, as the spontaneous permeation rate would be too low to gain sufficient statistics on simulation timescales. In addition, the driving force required in nonequilibrium simulations in terms of, e.g., a concentration gradient or an applied voltage would be unrealistically high.

Cross-References

- ▶ Potassium Channel Selectivity and Gating at the Selectivity Filter: Structural Basis
- ▶ Potassium Channels: Their Physiological and Molecular Diversity

References

Berneche S, Roux B. Energetics of ion conduction through the K^+ channel. Nature. 2001;414:73–7.

Chipot C, Pohorille A, editors. Free energy calculations, Springer series in chemical physics, vol. 86. New York: Springer; 2007.

de Groot BL, Grubmüller H. The dynamics and energetics of water permeation and proton exclusion in aquaporins. Curr Opin Struct Biol. 2005;15:176–83.

de Groot BL, Peter Tieleman D, Pohl P, Grubmüller H. Water permeation through gramicidin A: desformylation and the double helix; a molecular dynamics study. Biophys J. 2002;82:2934–42.

Doyle DA, Morais Cabral J, Pfuetzner RA, et al. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science. 1998;280(5360):69–77.

Hille B. Ion channels of excitable membranes. 3rd ed. Sunderland: Sinauer; 2001. ISBN 0-87893-321-2.

Hummer G, Rasaiah JC, Noworyta JP. Water conduction through the hydrophobic channel of a carbon nanotube. Nature. 2001;414:188–90.

Kalra A, Garde S, Hummer G. Osmotic water transport through carbon nanotube membranes. Proc Natl Acad Sci USA. 2003;100(18):10175–80.

Kumar S, Rosenberg JM, Bouzida D, Swendsen RH, Kollman PA. The weighted histogram analysis method for free-energy calculations on biomolecules. I. The method. J Comput Chem. 1992;13(8):1011–21.

Kutzner C, Grubmüller H, de Groot BL, Zachariae U. Computational electrophysiology: the molecular dynamics of ion channel permeation and selectivity in atomistic detail. Biophys J. 2011;101:809–17.

Preston GM, Carroll TP, Guggino WB, Agre P. Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein. Science. 1992;256(5055):385–7.

Sachs JN, Crozier PS, Woolf TB. Atomistic simulations of biologically realistic transmembrane potential gradients. J Chem Phys. 2004;121:10847–51.

Suenaga A, Komeiji Y, Uebayasi M, Meguro T, Saito M, Yamato I. Computational observation of an ion permeation through a channel protein. Biosci Rep. 1998;18(1):39–48.

Torrie GM, Valleau JP. Nonphysical sampling distributions in Monte Carlo free-energy estimation: umbrella sampling. J Comput Phys. 1977;23(2):187–99.

Zhu F, Tajkhorshid E, Schulten K. Pressure-induced water transport in membrane channels studied by molecular dynamics. Biophys J. 2002;83:154–60.

FMN-Binding Fluorescent Proteins (FbFPs)

▶ Flavin Mononucleotide-Binding Fluorescent Proteins

Focused Ion Beam-SEM

▶ Electron Microscopy: Classical Sample Preparation

F_oF_1-ATP Synthase

▶ ATPase: Overview

Fold Recognition

▶ Homology Modeling of Protein Structures
▶ Protein Structure Prediction and Structural Annotation of Proteomes

Force-Fluorescence Spectroscopy

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Synonyms

Single-Molecule Methods

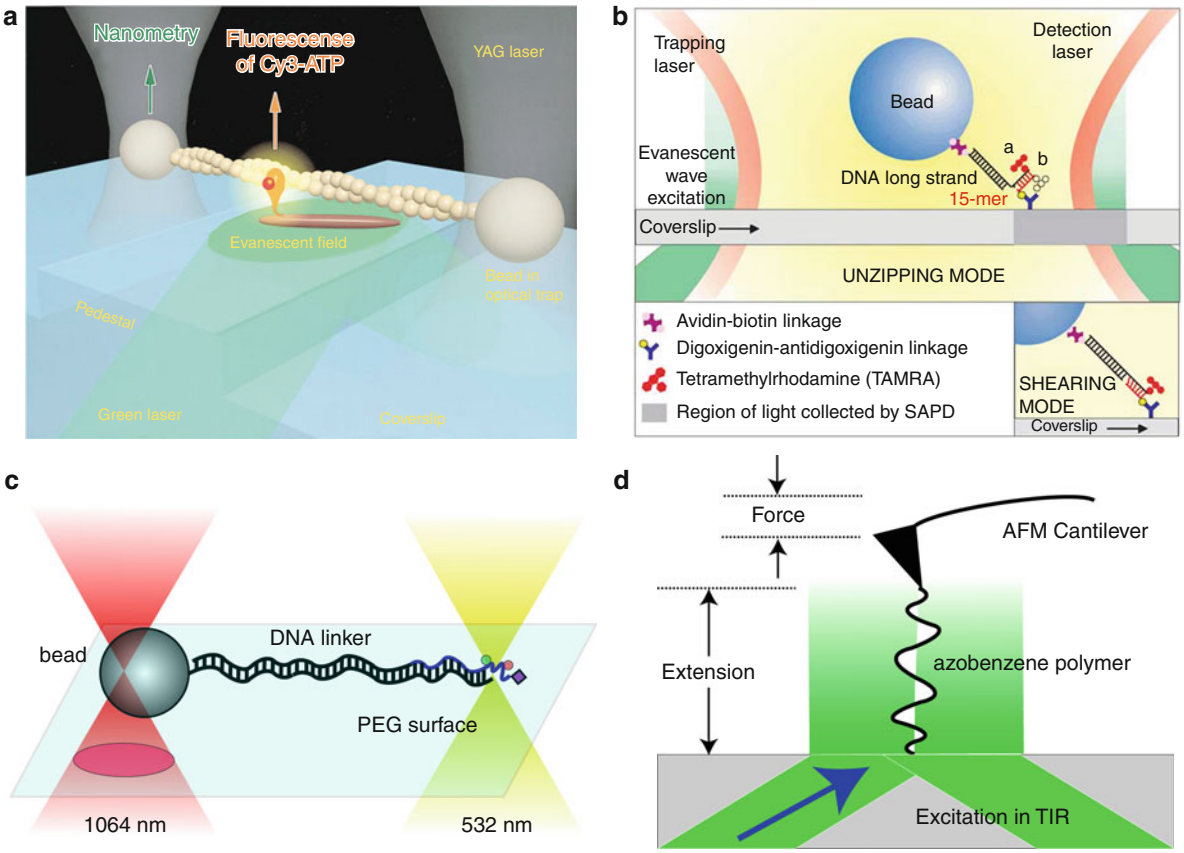
Definition

Force-fluorescence spectroscopy generally refers to the combination of single-molecule manipulation technique with simultaneous single-molecule fluorescence measurement to characterize the mechanochemical properties of a macromolecule.

Basic Characteristics

On the one hand, single-molecule manipulation using scanning probe techniques, ▶ optical tweezers, and ▶ magnetic tweezers enables mechanical perturbation of a system at the single-molecule level. On the other hand, intermediates in time-dependent reactions that are otherwise difficult to study in conventional ensemble experiments due to the averaging over many molecules can now be observed and studied directly using single-molecule fluorescence techniques. The combination of single-molecule manipulation techniques with single-molecule fluorescence measurements can thus find many potential applications in biophysical research, for example, manipulation of a macromolecule, while observing conformational transitions of the molecule in real time. ▶ Optical tweezers, ▶ magnetic tweezers, and ▶ atomic-force microscopy have all been combined with fluorescence microscopy for simultaneous manipulation and fluorescence measurements at the single-molecule level.

One of the first studies of this kind was targeting single myosin molecules and their interactions with actin (Ishijima et al. 1998). A single actin filament with beads attached to both ends was levitated in solution using optical tweezers (Fig. 1a). The single actin filament was brought into contact with a single myosin molecule immobilized on the surface of a coverslip. Binding of a single myosin molecule to the actin filament in the presence of ATP triggers the displacement of the actin filament, which was



Force-Fluorescence Spectroscopy, Fig. 1 Schematics of various force-fluorescence experiments at the single-molecule level

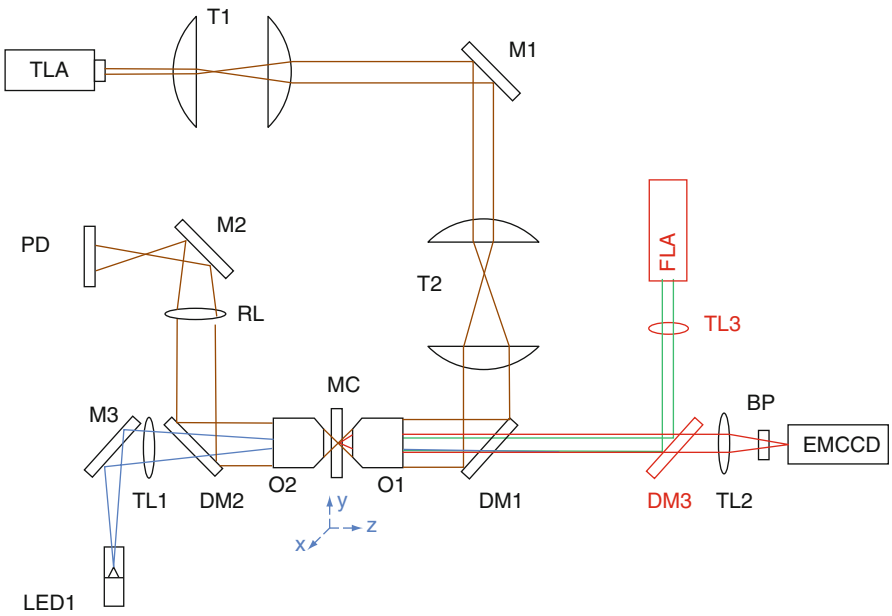
measured by ▶ optical tweezers. Simultaneously, individual ATPase reactions of the myosin motor were monitored as the time course of binding and unbinding of a Cy3-labeled ATP analogue using ▶ total-internal-reflection fluorescence (TIRF) microscopy. The unique advantage of this experiment is that the temporal relationship between force generation and ATP hydrolysis by the motor can be directly measured. The authors found that force generation does not always coincide with the release of bound nucleotide. Instead, there is a frequent delay of several hundreds of milliseconds after release of the bound nucleotide before the displacement of the actin. However, it is widely accepted that the force generation in myosin is directly coupled to the release of ADP. These findings contradict this view and suggest the presence of a memory state in single molecules of myosin.

In a different setup combining ▶ optical tweezers with ▶ TIRF microscopy (Lang et al. 2004), Lang et al. applied mechanical force to a single DNA molecule immobilized on a surface using optical tweezers, while simultaneously measuring the dissociation of a DNA strand marked by fluorescence labels. In this experimental design (Fig. 1b), optical trapping and single-molecule fluorescence coincide also in space. The challenges arising from this design are the photobleaching of fluorophores by the intense trapping laser through multiphoton processes and the selective detection of photons emitted by the single fluorophores of interest. Careful choice of fluorophores and engineering of the microscope, including alternating fluorescence excitation and optical trapping, are necessary to overcome these challenges (Lang et al. 2004). This design will be useful for certain systems where it is difficult to separate spatially the point of manipulation from the point of fluorescence excitation and detection.

Confocal fluorescence imaging was also combined with optical tweezers to study the effect of subpiconewton forces on the conformational dynamics of Holiday junctions (Hohng et al. 2007). The Holiday junction is a crossover structure that can be formed by two double-stranded DNA molecules. It is an important intermediate in DNA homologous recombination. In this experiment (Fig. 1c), individual Holiday junctions were immobilized on a coverslip surface. One arm of the Holiday junction was attached to a long double-stranded DNA linker through complementary base pairing. The other end of the linker was bound to the surface of a bead held in solution by optical tweezers, through which force can be exerted and transmitted through the DNA linker to change the conformation of the Holiday junction. Force and single-molecule fluorescence were measured simultaneously but separated in space. The conformational fluctuations of the Holiday junction under the influence of the mechanical force were exquisitely monitored through ▶ fluorescence resonance energy transfer measurements. In addition, these results demonstrated a lever-arm effect, where the response of the molecule to mechanical force can be amplified with an increase in the molecular "arm" length.

For certain types of optical tweezers, the optical setup can be easily modified to incorporate epi-fluorescence imaging with simultaneous ▶ optical trapping (see this volume on ▶ Optical Tweezers). This principle was demonstrated by Wuite and coworkers (van Mameren et al. 2009), and Chemla and coworkers (Min et al. 2009). In one experiment, the disassembly of Rad51 proteins from preformed nucleoprotein filament held under tension was measured (van Mameren et al. 2009). The disassembly of Rad51 protein from nucleoprotein filament is necessary for the completion of eukaryotic homologous recombination and was monitored using fluorescently labeled Rad51 in vitro. Van Mameren et al. found that this process can slow down and even be stalled upon application of tension to the filament. In a different experiment (Min et al. 2009), epi-fluorescence was used to image a fluorescently labeled single bacterium trapped by optical tweezers. The authors measured bacterial flagellar rotation using back focal plane interferometry, which offers a temporal resolution greater than 100 Hz.

▶ Atomic-force microscopy (AFM) and ▶ magnetic tweezers were also combined with TIRF to measure conformations of macromolecules in response to mechanical force. In one study (Hugel et al. 2002), a single azobenzene polymer was stretched between an AFM cantilever and a flint glass surface. The polymer conformation is sensitive to 365-nm light input due to the presence of the azobenzene group. Excitation of the azobenzene group by 365-nm light delivered through TIRF (Fig. 1d) triggers the reversible *cis-trans* isomerization and, in turn, changes the extension of the molecule, which was monitored using AFM. This elegant example demonstrated for the first time a single-molecule device that is capable of optomechanical energy conversion. In a second example (Kufer et al. 2008), AFM was used to pick up individual DNA



Force-Fluorescence Spectroscopy, Fig. 2 Schematic design of an optical tweezers with simultaneous epi-fluorescence imaging. *TLA* trapping laser; *T*1 and *T*2 telescopes; *M*1, *M*2, and *M*3 mirrors; *DM*1, *DM*2, and *DM*3 dichroic mirrors; *O*1 and *O*2 objectives; *MC* microfluidic chamber; *TL*1, *TL*2, and *TL*3 tube lens; *RL* relay lens; *PD* photodetector; LED1 light source for bright field imaging; *FLA* fluorescence excitation laser; *BP* band-pass filter; *EMCCD* electron-multiplying CCD camera for fluorescence imaging

oligonucleotide molecules from a surface for ordered assembly of these molecules at a distant location. TIRF was used to characterize the quality and efficiency of this process. Also, magnetic tweezers have been combined with TIRF to study the DNA packaging motor of bacteriophage phi29 (Hugel et al. 2007).

All these hybrid techniques detect fluorescence from single-photon excitation process. A majorities of them has not been applied to live cells. One potential future application of force-fluorescence spectroscopy is to study single-molecule activity in the context of a live cell, for example, manipulation of a single receptor on the cell surface and measurement of reporter gene expression to probe the mechanisms of mechanotransduction. Compared to single-photon excitation, two-photon excited (TPE) fluorescence has the superior advantages of low background, intrinsic three-dimensional (3D) resolution, and reduced overall phototoxicity for biological samples. Therefore, TPE may be well suited for integration with single-molecule manipulation for simultaneous fluorescence detection with high sensitivity. This could be very useful for systems that are typically studied deep in solution or tissue, where evanescent field excitation/TIRF is difficult. Custom-built microscopes that combine angstrom resolution optical tweezers with TPE fluorescence are just beginning to appear (Cheng et al. 2010), in which a 830-nm trapping laser is used for simultaneous TPE excitation and optical trapping.

The engineering aspects of force-fluorescence microscope are more complex than a conventional force-measuring technique because of the need to measure both force and fluorescence simultaneously. However, epi-fluorescence detection can always be incorporated into an existing ▶ optical tweezers as shown in Fig. 2. A potential drawback is the high fluorescence background that may not allow detection of fluorescence at a single-molecule level. Further engineering designs, for example, incorporation of TIRF excitation, confocal excitation, or TPE excitation are necessary to guarantee fluorescence detection at the single-molecule level. It is very likely that depending on the system to be studied and the experimental geometry required, different fluorescence excitation schemes will offer different advantages. For certain cases, force and fluorescence measurements are desired to coincide in space. The design by Block and coworkers will be suitable for this purpose, with further developments possible. In other cases where trapping and fluorescence detection can be separated in space, alternative schemes can be designed and used (Hohng et al. 2007; Ishijima et al. 1998).

With the development of ▶ nanometer resolution fluorescence detection (Yildiz et al. 2003) and subnanometer measurement by ▶ optical tweezers, force-fluorescence spectroscopy is anticipated to reach higher resolution for both manipulation and fluorescence detection, which offers a great tool for

biophysicist to understand the fundamental mechanochemistry of macromolecules as well as mechanotransductions that occur in the cell.

Cross-References

- ▶ Atomic Force Spectroscopy
- ▶ Fluorescence Labeling of Nucleic Acids
- ▶ Magnetic Tweezers
- ▶ Optical Tweezers
- ▶ Protein Fluorescent Dye Labeling
- ▶ Single Fluorophores Photobleaching
- ▶ Single-Molecule Fluorescence Resonance Energy Transfer
- ▶ TIRF

References

Cheng W, Hou X, Ye F. Use of tapered amplifier diode laser for biological-friendly high-resolution optical trapping. Opt Lett. 2010;35:2988–90.

Hohng S, Zhou R, Nahas MK, Yu J, Schulten K, Lilley DM, Ha T. Fluorescence-force spectroscopy maps two-dimensional reaction landscape of the holliday junction. Science. 2007;318:279–83.

Hugel T, Holland NB, Cattani A, Moroder L, Seitz M, Gaub HE. Single-molecule optomechanical cycle. Science. 2002;296:1103–6.

Hugel T, Michaelis J, Hetherington CL, Jardine PJ, Grimes S, Walter JM, Falk W, Anderson DL, Bustamante C. Experimental test of connector rotation during DNA packaging into bacteriophage phi29 capsids. PLoS Biol. 2007;5:e59.

Ishijima A, Kojima H, Funatsu T, Tokunaga M, Higuchi H, Tanaka H, Yanagida T. Simultaneous observation of individual ATPase and mechanical events by a single myosin molecule during interaction with actin. Cell. 1998;92:161–71.

Kufer SK, Puchner EM, Gumpp H, Liedl T, Gaub HE. Single-molecule cut-and-paste surface assembly. Science. 2008;319:594–6.

Lang MJ, Fordyce PM, Engh AM, Neuman KC, Block SM. Simultaneous, coincident optical trapping and single-molecule fluorescence. Nat Meth. 2004;1:133–9.

Min TL, Mears PJ, Chubiz LM, Rao CV, Golding I, Chemla YR. High-resolution, long-term characterization of bacterial motility using optical tweezers. Nat Meth. 2009;6:831–5.

van Mameren J, Modesti M, Kanaar R, Wyman C, Peterman EJ, Wuite GJ. Counting RAD51 proteins disassembling from nucleoprotein filaments under tension. Nature. 2009;457:745–8.

Yildiz A, Forkey JN, McKinney SA, Ha T, Goldman YE, Selvin PR. Myosin V walks hand-over-hand: single fluorophore imaging with 1.5-nm localization. Science. 2003;300:2061–5.

Fourier Transform Infrared Photoacoustic Spectroscopy (FTIR-PAS)

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Synonyms

Optoacoustic spectroscopy; Photoacoustic spectroscopy; Photothermal spectroscopy

Definition

▶ FTIR-PAS is a unique extension of IR spectroscopy which combines the utility of interferometry with the standard sample-gas microphone of the photothermal technique for depth-profile analysis of materials. FTIR-PAS is the most powerful spectroscopy-based depth-profiling tool that enables nondestructive and noncontact measurements with minimal sample preparation need.

Although absorption spectrum is retrieved from FTIR-PAS experiments, the thermal behavior of the sample rather than the optical properties plays a major role in the generation of PA signal.

Basic Characteristics

The evolution of photoacoustic spectroscopy (PAS) began with the discovery of emission of sound from a thin diaphragm exposed to modulated (mechanically chopped) sunlight by Alexander Graham Bell in 1880. However, the concept was impractical until the advent of microphone in the 1930s. Similar effect could be observed when infrared or ultraviolet light is used. Unlike most spectroscopic techniques, PAS is not based on the measurement of electromagnetic radiation. Rather, PAS involves measurement of acoustic