Optical Tweezers

Methods and Protocols

Edited by

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Preface

Numerous biological processes are dependent on the minute forces and displacements generated by enzymes capable of converting chemical energy into mechanical motion. For example, DNA and RNA polymerases generate forces during DNA synthesis and transcription, while the microtubule motors kinesin and cytoplasmic dynein generate forces that regulate spindle and chromosome positioning during mitosis. Deciphering the molecular mechanisms of these mechanoenzymes has fascinated scientists for more than 150 years since the discovery of muscle myosin. However, it was the invention of optical tweezers in 1986 by Arthur Ashkin that gave researchers the ability to investigate the force-generating molecular mechanisms of single mechanoenzymes. Optical tweezers, which can measure forces of 0.01 to ~1000 pN, have been invaluable in defining the forces and displacements that these biological nanomachines generate. While the optical tweezers of the past have been largely custom-built and used by biophysicists, they are now being more widely employed by nonexperts, owing to a more detailed description of optical tweezers systems and the availability of commercial solutions. However, a thorough description of the theory and design, together with protocols for the calibration and application of optical tweezers to biological systems both in vitro and in vivo, had not until now been compiled into a single resource.

The aim of this volume is to provide a comprehensive overview of optical tweezers setups, both in practical and theoretical terms, to help biophysicists, biochemists, and cell biologists alike to build and calibrate their own instruments and to perform force measurements on mechanoenzymes both in isolation in vitro and in living cells. To aid the reader, this volume has been divided in three parts. The chapters in Part I present the theory and practical design of optical tweezers both without and in combination with single-molecule fluorescence imaging as well as instructions for calibrating and stabilizing optical tweezers. Part II provides detailed protocols for performing force measurements on single DNA- and microtubule/actin-associated mechanoenzymes in isolation as well as protocols for protein unfolding/refolding experiments and the study of protein degradation. Part III describes the recent advances that have opened up quantitative force measurements on actin and microtubule motors in living cells.

It is my hope that, in addition to aiding seasoned users of optical tweezers, this volume will help to further expand the accessibility and use of optical traps by scientists of diverse disciplines. In doing so, may it foster new creative and collaborative approaches for using these exquisitely sensitive instruments to understand how molecular machines in the cell generate force and motion.

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