

# Atomic Force Microscopy in Structural and Functional Studies of Biomolecules

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## Abstract

Atomic force microscopy (AFM) has emerged as a cornerstone technology in molecular and cellular biophysics, offering nanometer-scale resolution under near-physiological conditions. Unlike electron microscopy (EM), AFM preserves native biomolecular states and enables simultaneous acquisition of topographical and mechanical data. This review synthesizes the principles, applications, methodological advances, and future directions of AFM in both structural and functional studies of biomolecules. Structural applications include high-resolution imaging of proteins, nucleic acids, and membrane systems, while functional investigations leverage single-molecule force spectroscopy (SMFS), binding interaction analysis, and mechanobiology assays. Methodological innovations such as high-speed AFM (HS-AFM), functionalized probes, and correlative multi-modal techniques have expanded AFM's capacity to probe dynamic processes in real time. Despite challenges such as tip-induced artefacts, throughput limitations, and data interpretation complexities, emerging trends point toward AI-assisted image analysis, in-cell AFM, and integration with molecular simulations. Functionalized nanosensors and automated platforms promise to transform AFM from a specialized research instrument into a high-throughput, intelligent biophysical tool. By bridging the gap between structural detail and functional insight, AFM is poised to play a pivotal role in advancing our understanding of biomolecular mechanisms, disease pathology, and therapeutic development.