Preface: Special Topic on Single-Molecule Biophysics

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Single-molecule measurements are now almost routinely used to study biological systems and processes. The scope of this special topic emphasizes the physics side of single-molecule observations, with the goal of highlighting new developments in physical techniques as well as conceptual insights that single-molecule measurements bring to biophysics. This issue also comprises recent advances in theoretical physical models of single-molecule phenomena, interpretation of single-molecule signals, and fundamental areas of statistical mechanics that are related to single-molecule observations. A particular goal is to illustrate the increasing synergy between theory, simulation, and experiment in single-molecule biophysics. *Published by AIP Publishing.* https://doi.org/10.1063/1.5028275

From the initial optical detection of single molecules in the condensed phase almost 30 years ago,[1–4] it took a remarkably short time to the first applications in biological systems.[5] Breakthroughs of optical techniques such as single-molecule Förster resonance energy transfer (FRET)[6] were soon joined by single-molecule mechanics,[7–11] and by the turn of the century, it was obvious that these methods would become indispensable for understanding the physical behavior of biological systems. The practical and conceptual challenges arising from those advances triggered parallel developments in theory—from fundamental aspects in statistical mechanics to a rapidly growing toolbox of sophisticated data analysis techniques.[12] Simulations, with their traditional affinity for individual molecules, became a natural partner early on.[13] The continued improvements in experimental time resolution on the one hand and the rapidly increasing computational power and progress in simulation techniques on the other have led to a growing overlap of accessible time scales. Consequently, single-molecule experiments are now regular benchmarks for simulations, and, in return, simulations are an indispensable resource for interpreting and guiding experiments.

One key goal of this special topic on single-molecule biophysics is to illustrate the intense synergy between experiment, theory, and simulation that has resulted from these developments. Indeed, the majority of articles in this issue take advantage of such multidisciplinarity. To a significant extent, it is this mutual fertilization that has enabled the field to maintain its remarkably high level of technical and conceptual innovation. At the same time, many single-molecule methods are now well-established and integral parts of diverse areas of research. Especially in biology, methods such as single-molecule FRET or superresolution microscopy have, in many respects, joined the ranks of standard techniques and enable systems of growing diversity and increasing complexity to be investigated. The focus of this special topic is to provide, from a physics perspective, a cross section of current developments in techniques as well as conceptual insights that single-molecule measurements enable. To our great pleasure, an impressive group of authors has contributed to our effort, among them are some of the true pioneers of the field.

An essential driving force for progress in single-molecule biophysics has been the development of new instrumentation and experimental techniques, and correspondingly strong is the representation of this aspect. The impressive contributions in this issue include diffusometry based on anti-Brownian electrokinetic trapping that enables investigation of the assembly dynamics of large protein complexes,[14] sensitive charge measurements of single molecules in nanofabricated traps,[15] plasmonic near-field enhancement of fluorescence,[16] multiplexed single-molecule FRET with 48-spot confocal detection to greatly enhance throughput,[17] high-pressure single-molecule FRET for the study of protein folding,[18] *in situ* temperature monitoring combined with single-molecule FRET,[19] integrated confocal and widefield single-molecule detection,[20] phasor-based 3D localization microscopy,[21] ultra-high-speed/high precision[22] and high-specificity[23] AFM-based force experiments, and the integration of optical tweezers with fluorescence polarization.[24]

Closely connected to new experimental methods are developments in data analysis and interpretation, a key component of quantitative single-molecule measurements. As a classic strength of single-molecule biophysics, probing the kinetics and dynamics of biomolecules takes center stage. An astonishing range of approaches is being developed to extract as much information as possible from experiments, from the shape analysis of transfer efficiency distributions,[25] change point analysis,[26] hidden Markov models,[27–29] and cluster analysis[30] to model selection by Bayesian nonparametrics[31] and unsupervised learning and rate distortion theory.[32] Another important theme is the subpopulation-specific

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distance information available from single-molecule FRET. Increasingly detailed structural information is becoming available from the global analysis of transfer efficiency histograms[33] and from structural analysis based on Bayesian parameter estimation;[34] single molecule studies begin to reveal structural information even for highly dynamic and complex molecular systems.[29] Inferring the distance distributions in unfolded and intrinsically disordered proteins greatly benefits from atomistic[35] and coarse-grained[36] molecular simulations combined with polymer models.[35–37]

As single-molecule measurements offer increased information about conformational ensembles sampled by individual biomolecules, theory and simulations often play a key role in pinning down the underlying physical picture and/or molecular details.[38] For example, theoretical studies indicate a surprising force-induced rupture mechanism involving two transition states in cell adhesion complexes,[39] and a comprehensive picture of the energetics underlying self-assembly of alpha-synuclein emerges from a combination of high-speed atomic force microscopy combined with computer simulations.[40] Important insights about the effect of the charge distribution along a peptide chain on compactness of intrinsically disordered proteins[41] and their dynamics modulated by internal friction[37] are obtained through polymer theory.

A new and increasingly important topic of theoretical and experimental investigations is the interface between the individual molecule of interest and the instrument used to interrogate this molecule. For example, observation of transition paths of biomolecular folding[42] pushes the time-resolution limits of single-molecule force spectroscopy;[43] as a result, the dynamic coupling between the (unobservable directly) molecular extension and the (observable) displacement of the force probe must be explicitly incorporated by a proper theoretical analysis of the experiment.[44] Likewise, ultralow force measurements of biopolymer elasticity accomplished by magnetic tweezers are affected by the excluded volume effect imposed by the tethering surfaces; proper interpretation of such measurements thus forces one to study, theoretically, the statistical-mechanical problem of polymer chains in the presence of tethering-induced confinement.[45] Artifacts introduced by force feedback need to be carefully considered when extracting the underlying parameters of the free-energy landscapes from single-molecule force spectroscopy measurements,[46] and solvent effects can impact single-molecule FRET measurements.[47]

Equipped with this broad foundation of techniques and concepts, single-molecule methods and ideas are now being applied to a rapidly growing range of topics and systems, which include the dynamics of lipid bilayers,[48] nucleosome assembly,[49] collective dynamics of molecular motors,[50] biophysics and function of large multimeric proteins,[51] and even embryonal development.[52]

In summary, single-molecule techniques offer an increasingly versatile toolkit for probing a wide variety of complex physical phenomena occurring in living organisms. At the same time, single-molecule studies often provide conceptual insights into molecular kinetics, polymer physics, protein and nucleic acid folding, nonequilibrium statistical mechanics, and other fundamental disciplines. This special topic showcases many examples of recent advances and illustrates the synergy of experiment, theory, and simulations that has been essential for much of the progress and impact of single-molecule biophysics.

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