Available online at www.sciencedirect.com

ScienceDirect

Physics of Life Reviews 13 (2015) 107–137

PHYSICS of LIFE reviews

www.elsevier.com/locate/plrev

Review

Extracting physics of life at the molecular level: A review of single-molecule data analyses

Warren Colomb, Susanta K. Sarkar *

Department of Physics, Colorado School of Mines, Golden, CO 80401, United States

Received 8 January 2015; accepted 9 January 2015

Available online 14 January 2015

Communicated by E. Shakhnovich

Abstract

Studying individual biomolecules at the single-molecule level has proved very insightful recently. Single-molecule experiments allow us to probe both the equilibrium and nonequilibrium properties as well as make quantitative connections with ensemble experiments and equilibrium thermodynamics. However, it is important to be careful about the analysis of single-molecule data because of the noise present and the lack of theoretical framework for processes far away from equilibrium. Biomolecular motion, whether it is free in solution, on a substrate, or under force, involves thermal fluctuations in varying degrees, which makes the motion noisy. In addition, the noise from the experimental setup makes it even more complex. The details of biologically relevant interactions, conformational dynamics, and activities are hidden in the noisy single-molecule data. As such, extracting biological insights from noisy data is still an active area of research. In this review, we will focus on analyzing both fluorescence-based and force-based single-molecule experiments and gaining biological insights at the single-molecule level. Inherently nonequilibrium nature of biological processes will be highlighted. Simulated trajectories of biomolecular diffusion will be used to compare and validate various analysis techniques.

Keywords: Single molecule data analysis

Contents

* Corresponding author.
E-mail address: ssarkar@mines.edu (S.K. Sarkar).

http://dx.doi.org/10.1016/j.plrev.2015.01.017

1. Introduction

We think, speak, and write about science at the single-molecule level, yet we have been limited to do ensemble experiments until single-molecule studies began over 20 years ago. Today, single-molecule techniques are widely used in many fields, most notably biology. Single-molecule studies offer insight into molecular dynamics that are normally hidden in the statistics of a bulk measurement. It is now possible to gain unprecedented insights into detailed enzyme kinetics [1–5], rare and transient events [6,7], static and dynamic heterogeneity [8–12], conformational dynamics [13–16] and memory [17], kinetics on extended substrates [18–27], protein folding kinetics [28–32], single-molecule counting and stoichiometry [33–36], experimental verifications of fundamentals of thermodynamics and statistical mechanics [37–49], strength of chemical bonds [50], and potential energy landscapes [51–54]. Depending on the single-molecule technique, it is now possible to perform experiments with sub-angstrom spatial resolution, nanosecond temporal resolution, and 0.01 pN force resolution at different solution conditions and temperatures. These abilities, and general areas of study can be utilized in chemistry, physics, biology, biotechnology, and materials science; therefore, single-molecule fluorescent assays are very important tools for a wide range of studies in multiple fields of research.

2. Brief history of single-molecule study

In 1961, Rotman reported measuring signal from individual β–D-galactosidase using 6HFG as fluorescent markers [55]. In 1976, Hirschfeld reported observing single γ globulin molecules tagged with 80–100 fluorescein isothiocyanate dyes via polyethyleneimine using total internal reflection excitation [56]; Neher and Sakmann reported current through single ion channels associated with acetylcholine receptor [57]. In 1980, a single barium ion (Ba^+) was observed in a Paul RF quadrupole trap at room temperature [58]. In 1982, atomic resolution characterization of a surface by scanning tunneling microscope (STM) was reported [59], followed by atomic force microscope (AFM) in 1986 [60]. In 1986, observation of single particles with optical trap was reported [61]. The first detection of single fluorescent molecules was reported by using absorption [62], in 1989, and fluorescence excitation spectroscopy [63], in 1990, in a solid at liquid helium temperatures. In these studies, fluorescent pentacene molecules were doped in p-terphenyl.