Preface

Steady progress in experimental techniques and imaging capabilities has made the observation of individual atoms and molecules a routine practice in many laboratories worldwide. Despite this fact, single-molecule measurements remain difficult to perform and interpret. The exceedingly weak signals arising from individual molecules are more readily degraded and distorted by macroscopic-scale noise sources than are most ensemble averaged signals. We accept these difficulties since single-molecule experiments offer qualitatively new information about the behavior of physical systems, information unobtainable by traditional means. Observations of individual molecules, nanoparticles and other nano-objects reveal the full extent of their heterogeneity, be it for enzymes at physiological conditions, or for fluorescent probes in supercooled molecular liquids. Even more importantly, single-molecule signals open a window to directly observe dynamical fluctuations at the nanoscale.

A variety of modern techniques providing single-molecule sensitivity have been reviewed in the literature. The first methods to observe single molecules were scanning probe microscopies: STM (scanning tunnelling microscopy)[1] and AFM (atomic force microscopy).[2] In biophysics, electrical conductivity measurements can monitor the function of single ion-channels.[3] More recently, optical spectroscopy and fluorescence microscopy[4,5] have reached single-molecule sensitivity. In spite of the differences in the experimental approaches, the obtained single-molecule data share the same characteristic features; the data is noisy and contains large and irreproducible fluctuations. Depending on the experiment, these fluctuations may be of primary interest or may frustratingly serve to obscure a more interesting dynamics. Stated differently, single-molecule data streams are inherently stochastic — a given single molecule data

trajectory is apparently random and is irreproducible. However, as we may extract statistically meaningful information from these data streams, their fundamental irreproducibility is an asset as well as a curse. While complex data treatments, analysis methods, statistical indicators, etc., are required to ensure the robustness of our conclusions, the information gained is unique and is inaccessible by ensemble-averaged measurements.

Statistical methods of data analysis applied to single molecules should, to the extent possible, be unbiased and model-free. They should eliminate the effects of noise, while retaining the significant features essential to understanding molecular dynamics. A number of procedures are currently in use to aid in this analysis, as summarized in Refs. 6 and 7. The simplest way to evaluate the time-trace of a single-molecule signal is to bin the signals by a low-frequency pass filter, and compare the average signal value to thresholds. This works well for slow variations, but the choice of bins and thresholds is somewhat arbitrary. More systematic analysis tools, either time-dependent (such as delay distributions, lifetimes, correlation functions) or signal-strength dependent (such as photon-counting histograms) do not involve any arbitrary parameter, but do average the data in ways which can sometimes erase the useful information. Finally, simple statistical indicators can sometimes answer a question about the data. Examples are the Mandel parameter indicating deviations from Poisson statistics in photon-counting traces, or indicators of the renewal character of series of events. (Is the data consistent with all events having been drawn from the same probability distribution?) Of course, an ideal analysis scheme would let the data "speak for itself", i.e. determine a model for the molecular dynamics based solely on the collected data without prior hypotheses influencing the final interpretation. This is seldom possible.

Since it is typically not possible to directly invert single-molecule data into a complete model for molecular dynamics, it is important to be able to model single-molecule experiments theoretically and computationally. Theoretical predictions for a given model may be compared to observables obtained experimentally, which allows for the refinement of hypothetical models via the scientific process. While most of the models currently used in the description of single-molecule experiments are based on traditional physical pictures (kinetics, diffusion, quantum mechanics, etc.), the observables