PREFACE

Theoretical and experimental breakthroughs are strongly coupled: major advances in fundamental theoretical concepts are often triggered by novel experimental methods and observations. Similarly, new theoretical ideas suggest more experiments. Single-molecule studies are a clear demonstration of this paradigm. The advent of optical single-molecule spectroscopy and atomic force microscopy has empowered novel experiments on individual biomolecules, opening up new frontiers in molecular and cell biology. And these experiments led to new theoretical approaches and insights. The single-molecule approaches offer unique insights not only for the distribution of molecular properties, but also for the dynamics of individual molecules information that cannot be provided by conventional ensemble averaged measurements.

In the past few years, important advances have been made in several areas where data from single-molecule experiments have provided fresh new perspectives. Driving these developments are questions including, for example, how proteins fold to specific conformations from the highly heterogeneous structures, how signal transductions take place on the molecular level, as well how proteins behave in membranes and in living cells. A general problem arising from these new experimental observations is the theoretical underpinning for the roles of fluctuations in biochemical reactions. For example: Why biological systems can robustly perform their functions even with the free energy gain or loss of the reactions being comparable to the thermal energy, $k_B T$?

With a strong conviction that the integration of experimental developments and theoretical advances is essential toward resolving these issues, we have organized two international conferences focusing on identifying and articulating these issues. The first conference was entitled, Linking Single Molecule Spectroscopy and Energy Landscape Perspectives, held on December 3, 2008, at the FUKUOKA convention center, Fukuoka, Japan (organized by T. Komatsuzaki and H. Yang) during the 46th Annual Conference of Biophysical Society of Japan. The second conference was entitled, New Approaches to Complexity of Protein Dynamics by Single Molecule Measurements: Experiments and Theories, from December 7 to 9, 2008, held at the Institute for Protein Research (IPR), Osaka University, Japan (organized by S. Takahashi, T. Komatsuzaki, M. Kawakami).

This volume consists of contributions from participants of the above-mentioned two conferences, including invited speakers, discussants, and organizers. The content of this volume is organized into two parts: one is experimental, and the other is theoretical development on single-molecule biophysics. Part I focuses mainly

to separate the time trace of photon trajectories in the diffusion process into the distance fluctuation between dye molecules and the other components (Gopich and Szabo, Chapter 10), a new theoretical framework to construct local equilibrium states and the corresponding multidimensional energy landscape from a scalar time series without a priori postulation of the concept of local equilibration and the detailed balance (Baba and Komatsuzaki, Chapter 11), a generalized Michaelis–Menten rate equation for nonequilibrium steady-state turnover reactions in which the original kinetic network model is mapped onto a flux network with unbalanced population currents and the concentration dependence of substrate for the average turnover rate derived (Wu and Cao, Chapter 12), a new construction scheme of a reduced form of the underlying multisubstate kinetic scheme for time trace when composed of two states, where the connections between the nodes in the network can have multiexponential waiting time probability density functions (Flomenbom, Chapter 13), a systematic survey of discrepancies found between numerical simulations and AFM experiments for mechanical unfolding of proteins including a caution of oversimplifications due to the projections of the intrinsic multidimensional free energy surface onto a low dimension (Yew, Olmsted, and Paci, Chapter 14), and the exploration of different types of mechanochemical coupling mechanisms (i.e., loose- and tight-coupling scenarios) functioning in myosin II in terms of the mean velocity and velocity fluctuation at various ATP concentration (Takagi and Nishikawa, Chapter 15).

We note, however, that the subject matter included herein should not be regarded as solved; rather, they indicate topics that have been identified for which solutions with various levels of completeness have been provided. It is therefore hoped that the ideas contained in this volume will motivate further innovations in both experiment and theory, and especially their closer interactions in the near future.

We finally acknowledge the financial supports for the above two conferences from the following organizations and programs: (1) Japan Science and Technology Agency, Promoting Globalization on Basic Research Programs, (2) Japan Science and Technology Agency, Core Research for Evolutional Science and Technology (CREST), and (3) The Institute for Protein Research, Osaka University.

T. KOMATSUZAKI
M. KAWAKAMI
S. TAKAHASHI
H. YANG
R. J. SILBEY

on three experimental approaches: single-molecule fluorescence based mainly on fluorescence resonance energy transfer (FRET), atomic force microscopy (AFM) and diffracted X-ray tracking (DXT), and their applications to important biological phenomena. This part begins with experimental investigations of protein folding and the development of a new fluorescence method for a long time detection without tethering proteins to glass (Takahashi and Kamagata, Chapter 1) and is followed by conformational and dynamic properties of unfolded proteins based on the confocal detection of single-molecule fluorescence (Nettels and Schuler, Chapter 2), and a quantitative analysis of FRET signals in terms of cumulative distribution functions for telomere DNA (Okamoto and Terazima, Chapter 3). The topic then turns to AFM, which is capable of measuring the mechanical response of a single molecule to an applied force. A systematic AFM study of the mechanical property of the unbinding force of biomolecular complexes and rigidity (stiffness) of various biomolecules are reviewed (Ikai, Afrin, and Sekiguchi, Chapter 4). A novel dynamic force spectroscopy using AFM is discussed, in which a stretched single molecule is driven by an external oscillatory motion and both the static and dynamic mechanical properties of the molecule can be obtained through the deflection of an AFM cantilever (Kawakami and Taniguchi, Chapter 5). A recent breakthrough in single-molecule experiments is the DXT method, which monitors the movements of individual nanocrystals linked to a specific site of proteins at the picometer scale (Sasaki, Chapter 6). The DXT method revealed a twisting motion involved in the gating dynamics of a membrane potassium channel, KcsA (Oiki et al. Chapter 7). Finally, an exploration of complex kinetics at the association/dissociation of epidermal growth factor receptor and Grb2 in cellular signal transductions using an in vitro reconstructed system was reviewed (Takagi, Morimatsu, and Sako, Chapter 8).

Part II focuses on the theoretical progress in single-molecule data analyses. In general, the observed time traces from single molecule are contaminated by several internal noises in addition to external noises arising from experimental settings. For example, the origin of the fluctuation in the FRET data ranges from photophysics, such as blinking and bleaching to different quantum yields of two dye molecules. It is therefore of crucial importance to recognize the existence of two distinct, but complementary stages, in the analyses of single-molecule time series: The first is of extracting the time trace of a desired physical quantity, such as an interdye distance from a noisy signal and the second is of constructing the underlying mechanisms from a scalar time series, such as multidimensional energy landscapes and networks composed of states in a kinetic scheme. Part II includes new theoretical frameworks to extract the scalar time trace of a desired physical quantity buried in a noisy time signal observed in single-molecule experiments, which are designed as an unbiased and quantitative interpretation of the data (Yang, Chapter 9), a comprehensive review of a theory of the FRET efficiency histograms obtained from single-molecule photon counting experiments, which is designed