Research Highlights

1. Combination of single-molecule FRET & optical tweezers

Understanding of the nature of the transition state is critical for reconstructing a reaction mechanism. Recently, it was predicted that the transition state of conformational dynamics can be studied by measuring the effect of mechanical force on reaction dynamics. This prediction was demonstrated in the study of RNA unfolding process by using optical tweezers, the most precise single-molecule manipulation tool, alone, but this method could be used only in a high force regime where the natural behavior of macromolecules were completely disrupted. Thus, to study the inherent properties, we need a new instrument which perturbs molecular behavior as little as possible. Single-molecule fluorescence resonance energy transfer (FRET) is a powerful tool for studying conformational dynamics at arbitrarily low forces and, therefore, there have been efforts to combine single-molecule FRET and optical tweezers. Prior attempts, however, exposed great technical difficulties. Detection of single-molecule fluorescence was difficult due to huge background signals coming from the strong trapping beam, and, furthermore, photobleaching of organic dyes was much more severe when the trapping beam and the excitation beam overlap.

By spatially separating trapping and excitation beams, we realized a combined single-molecule FRET and optical tweezers for the first time (Fig. 1(a)). As a model system, we studied DNA 4-way junction, or Holliday junction, which is known to switch between two conformers, isoI, and isoII. By applying different forces, we could control the behavior of the Holliday junction in a reversible manner (Fig. 1(b)); Holliday junction stays longer time in the isoI state at higher forces. Transition rates between isoI and isoII also change upon different applied forces. From the dependence of the transition rate on the applied force, we could find that the transition state is closer to the isoII state (Fig. 1(c)), which probably indicates that the breaking of stacking partners is the rate limiting step. Molecular dynamics simulation and supporting experiments are undergoing to test the hypothesis. We could also prove the lever arm principle at the single-molecule level. By doubling helical arm length, we could approximately double the force sensitivity of the Holliday junction (Fig. 1(d)).

We expect this new and exciting technique will open the door to exploring the inner workings of the cellular machines, allowing us to pull DNA, RNA, and proteins while watching their responses at the same time. In our laboratory, the combined setup is already being used to study the heterogeneity of hairpin ribozyme, the interaction between single-strand DNA and its binding proteins, helicase translocation along single-strand DNA, and mechanical stability of the DNA G-quadruplex.
2. Single-molecule 3-color FRET

Single-molecule FRET offers a direct means to study the conformational dynamics of biological molecules and intermolecular interaction. By labeling macromolecules with a donor and an acceptor and recording the time-dependent changes in energy transfer efficiency, we can track the distance changes between the two dyes in real time. In complex systems that are frequently found in biological interactions, however, coordinated movements of different segments are important, and we need a way to measure FRET among three spectrally distinct fluorophores to get a complete picture. In spite of the obvious necessity of 3-color FRET, its realization has been hindered due to contradictory requirements: a clear spectral separation of three fluorophore signals and an appreciable amount of FRET between them. For a clear signal separation, the spectral overlap should be small, but this leads to negligible FRET.

By selecting right fluorophores and optical filters, we realized single-molecule three-color FRET for the first time, extending the applicability of single-molecule FRET technique. As a model system, we labeled the DNA Holliday junction with three spectrally different dyes and observed correlated movements of different helical arms (Fig. 2). As a result, we proved that every helix is stacked all the time and that the parallel conformations are not populated. Now, using this unprecedented technique, diverse complex biological processes can be studied in single-molecule level. In our laboratory, this technique is being used to study different biological processes including helicase translocation on single strand DNA and DNA strand exchange mediated by RecA filament.

Reference

3. Suppression of quantum dot blinking & single quantum dot FRET

Colloidal semiconductor quantum dots have great potential as fluorescent markers for biological studies. Their outstanding brightness and photostability are promising for single-molecule fluorescence measurement in a living cell. Broad absorption and narrow emission spectra are ideal for multi-color imaging. Unfortunately, their potential for single-molecule studies was limited by the blinking that is universally observed from quantum dots, and thus blinking sets an intrinsic limitation to their usage. We discovered a way to almost completely suppress this blinking in ambient conditions. We found that passivation of the quantum dot surface with thiol moieties suppresses blinking with the emission duty cycle approaching 100% while maintaining bio-compatibility (Fig. 3). This discovery renders quantum dots ideal for single-molecule studies. As a proof of concept experiment, we
measured conformational dynamics of DNA Holliday junction using a quantum dot and an organic dye as a FRET pair. This was the first demonstration that single-molecule structural dynamics can be reliably probed by using semiconductor quantum dots. We hope our work will be an important step forward to the future of single-molecule fluorescence experiment where we can finally observe a variety of single-biomolecules doing their jobs in a living cell for unlimited time without worrying about background signal and blinking.

Reference

4. Role of Hfq in the translation regulation by sRNAs

Hfq is a global regulator protein in bacteria, which is required for the proper function of many noncoding small RNAs (sRNA). How Hfq helps sRNA regulate gene expression is under debate. We developed FRET assays to study how Hfq affects the interaction between a sRNA in E. coli, DsrA, and its regulation target mRNA, RpoS.

As a result, we observed that Hfq speeds up the annealing of DsrA and RpoS. The binding between the two RNAs, however, was transient due to the strand separation activity of Hfq, which was realized for the first time. We also found that Hfq also acts as an RNA chaperone by disrupting the stem loop of RpoS mRAN. Considering that DsrA needs to work in an emergency, we suspect that the ability of Hfq to make the sRNA/mRNA interaction more dynamic may be critical for timely action of sRNA. We expect our work will give insights into the working mechanism of other antisensor sRNAs requiring Hfq for their proper function.