# Protein Diffusion in Aqueous Solution for Revealing Spectrally Silent Conformation Change

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Since conformation changes of proteins and biomolecular interactions (including protein-DNA, or protein-protein interactions) are essential for biological functions, detections of these processes are important in chemistry and biochemistry to understand the reactions. For the detection of these processes, a variety of techniques have been developed. UV/vis absorption spectroscopy or emission spectroscopy are very powerful to trace the time development of reactions. However, these techniques have a limitation to detect the conformation changes of proteins and biomolecular interactions. Recently, our group discovered that the translational diffusion coefficient can be a useful and sensitive probe to detect the conformation change as well as the intermolecular interaction changes. Although many techniques, e.g., dynamic light scattering, Taylor dispersion, capillary method, NMR spectroscopy, have been developed to monitor molecular diffusion, molecular diffusion has never been considered as a time-dependent property during reactions. We have been developing a method based on the pulsed-laser induced transient grating (TG) technique to detect the time-dependent diffusion. Here, we report the time-resolved detection of protein conformation changes of a blue light sensor protein of phototropin by using the diffusion coefficient.

Phototropin (phot) is found in higher plants and green algae. It consists of N-terminal blue-light sensing domains, LOV1 and LOV2 (LOV= light, oxygen, and voltage sensor), and a serine-threonine kinase domain. The LOV1 and LOV2 domains bind a FMN molecule non-covalently. Previously, conformational changes of *Arabidopsis* (*At*) phot1-LOV2 with the linker (phot1-LOV2-linker) were investigated from the view point of the changes in the molecular diffusion coefficient by the time-resolved TG method. Although the absorption spectrum change completes within a few microseconds, the diffusion coefficient detected by the TG method decreased drastically with a time constant of 1.0 ms. This time-dependent diffusion coefficient was interpreted in terms of the unfolding of α-helices in the linker region. We extended this study to photochemical reactions of a variety of differently truncated constructs of a phot from *Chlamydomonas reinhardtii* (*Cr*) (LOV1, LOV1-hinge, LOV2, LOV2-linker and hinge-LOV2). We found that, in the dark state, LOV1 is in dynamic equilibrium between the monomer and dimer, and the main photochemical reaction is dimerization of the monomer and dissociation of the dimer. On the other hand, LOV1-hinge exists as the monomer and the photochemical reaction is the dimerization reaction associated with the unfolding of the helix of the hinge domain. The linker region including the Jα helix is rather stable upon photoexcitation. The helix of the hinge domain of hinge-LOV2 is slightly unfolded in the dark state and the major photoreaction is the dimerization event. These photochemical properties of *Cr* phot are considerably different from those of *At* phot. It should be noted that these conformation changes and the dimerization reactions cannot be detected by other optical methods, i.e., these processes are spectrally silent. These studies clearly demonstrate that the diffusion coefficient can be very useful for probing spectrally silent processes of proteins.