Supplementary Information

Time-resolved detection of light-induced conformational changes of heliorhodopsin

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SI-1. Transient absorption changes of *T*aHeR wild type (WT) and Y93G.

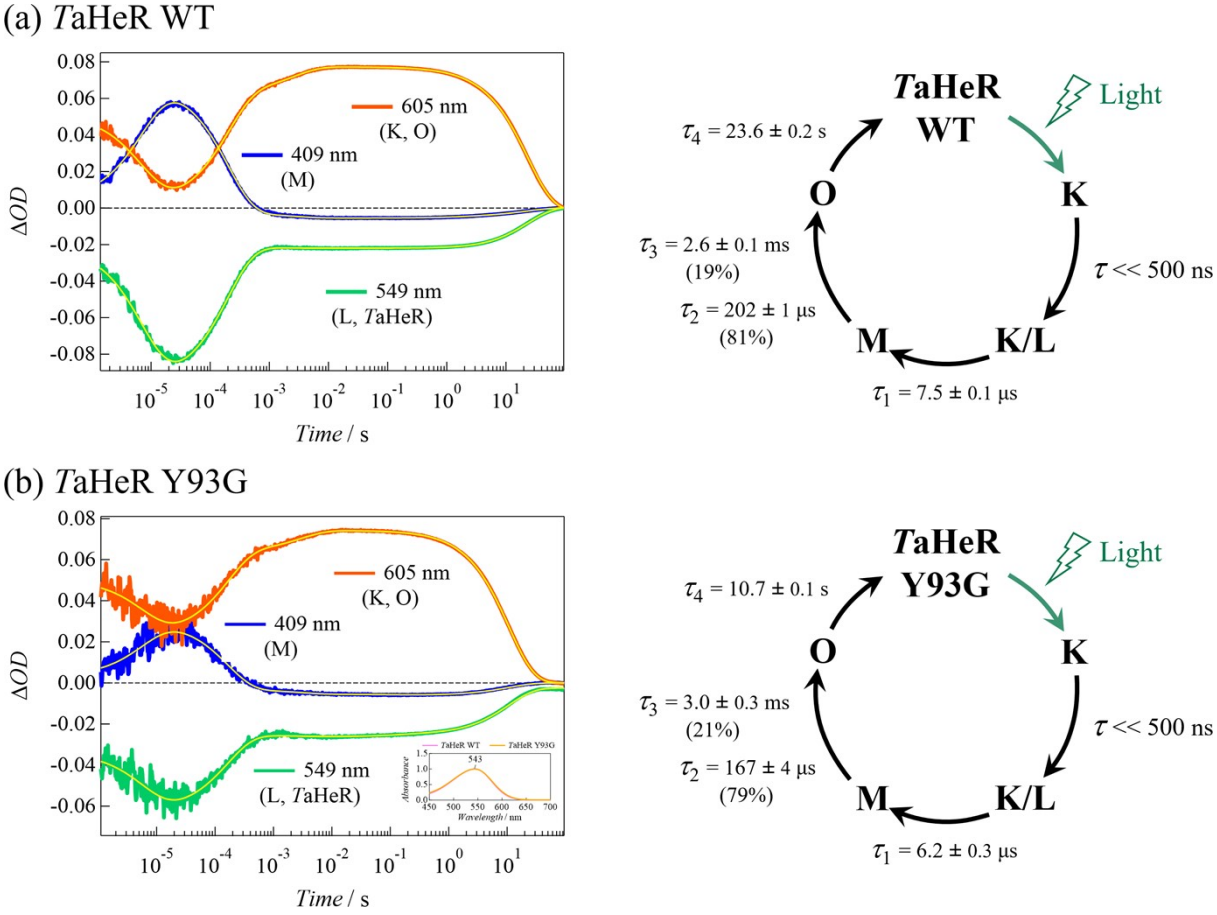

Figure S1. Transient absorption profiles (left) and the photocycles (right) of (a) *T*aHeR wild type (WT), and (b) Y93G. The proteins were solubilized in 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.05% DDM. The transient absorption signals were probed at 409, 549, and 605 nm to observe the accumulation of the M intermediate, the bleaching of the initial state/accumulation of the L intermediate, and the accumulation of the O intermediate, respectively. The lifetimes of intermediates in the photocycles were determined by the global fitting analyses of the transient absorption signals with a multi-exponential function (left, yellow lines). The decay of the M intermediate of *T*aHeR WT and Y93G exhibited two different lifetimes, and the percentages of the pre-exponential factors of each component in the amplitude of the full decay are indicated in parentheses. Inset: Absorption spectra of *T*aHeR WT (pink) and Y93G (orange).

SI-2. Concentration dependence on TG signals of *T*aHeR WT and Y93G.

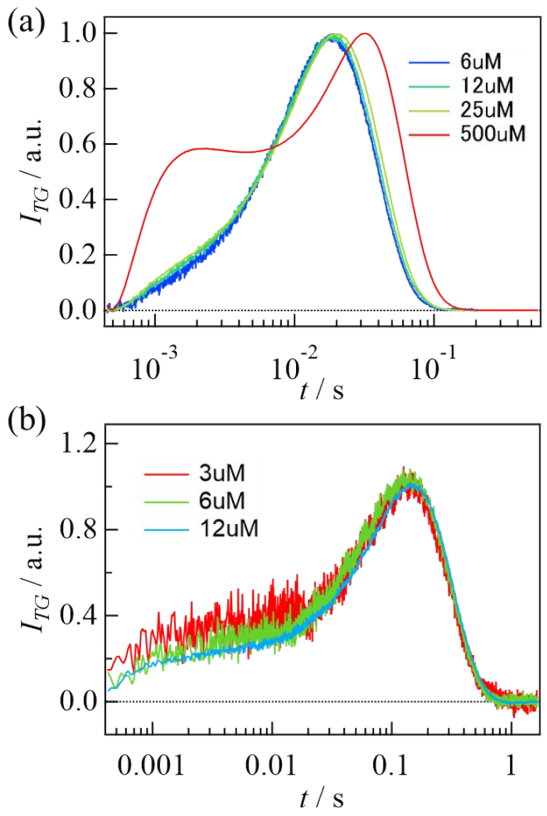

Figure S2. Concentration dependence of the diffusion signal of (a) *T*aHeR WT at q^2 of 9.9×10^{11} m^{-2} and (b) Y93G mutant at q^2 of 1.1×10^{11} m^{-2}. The signal intensities were normalized to the maximum intensity.

SI-3. Laser power dependence on the TG signals of *T*aHeR WT.

Since *T*aHeR exists as a dimer, the conformation of the dimer containing one light-adapted protomer and one dark-adapted protomer (LD dimer) and the dimer containing two light-adapted protomers (LL dimer) could be formed, and they could be different. We examined this possibility by monitoring the excitation light intensity dependence of the diffusion signal. Figure S3(a) shows the diffusion signal at various excitation light intensities in the range of 3.6–88 μJ/pulse. The square root of the TG signal intensity, which is proportional to the number of molecules undergoing *D* change, is plotted against the light intensity in Fig. S3(b). It increases linearly with the light intensity in the relatively weak light region and saturates above 40 μJ/pulse. If the *D* change requires the reaction of both protomers in the dimer, it should show quadratic behavior in the weak light region. The linear relationship indicates that the photoreaction of one protomer in the dimer leads to the *D* change.

When the diffusion signals in Fig. S3(a) are normalized by the peak intensities, they almost completely overlap as shown in Fig. S3(c), indicating that the amount of *D*-change is independent of the pulse light intensity. This light intensity independence is reasonable, if the conformation changes are the same between the LD and LL dimers. However, in this case, the signal intensity before the diffusion signal, which reflects the absorption change, should increase with increasing the population of LL dimer, since both protomers in a dimer should undergo the absorption change. Contrary to this expectation, the signal intensity before the diffusion signal also overlaps over a wide range of light intensities. On the basis of these observations, we conclude that the formation of the LL dimer is negligible.

The negligible formation of the LL dimer could be due to a small quantum yield of the reaction. According to a previous report, the relative isomerization quantum yield is 0.18 and 0.42 for *T*aHeR and bacteriorhodpsin (bR), respectively.[1] Because the absolute isomerization quantum yield of bR was reported to be 0.64,[2] the absolute isomerization quantum yield of *T*aHeR should be 0.27. Since the probability of the photoreaction of both protomers in the dimer is proportional to the square of the reaction yield, the yield of the LL dimer formation should be $(0.27)^2$=0.073, which is relatively small.

We also calculated accumulation of the photo-product by a multi-excitation within the excitation pulse as follows. Under our experimental conditions (excitation pulse energy: 3.3 - 88 μJ/pulse, pulse duration: 20 ns, wavelength: 532 nm, and irradiation area: 0.0078 cm^2), the photon fluence rate is calculated to be 5.8×10^{22} - 1.5×10^{24} cm^{-2} s^{-1}. Based on the molecular extinction coefficient (ε) of *T*aHeR, ε = 42,000 M^{-1} cm^{-1}, which is estimated by comparison between *T*aHeR absorption and retinal oxime absorption (ε = 33,900 M^{-1} cm^{-1}) produced by a hydrolysis reaction with hydroxylamine,[3, 4] the absorption cross-section of *T*aHeR is calculated to be 1.6×10^{-16} cm^2. Assuming that the concentration of *T*aHeR in the dark state is maintained during the pulsed light irradiation due to a rapid relaxation from the excited state, the maximum number of photons absorbed by each molecule within the pulse is calculated to be 0.19 - 4.8 depending on the pulse energy. If one *T*aHeR protein is photoexcited 4.8 times with a reaction quantum yield of 0.27, the long-lived photoproduct could be accumulated about 78% of the protein. In this case, the LL dimer of 61% could be produced. Although this is a maximum number from the above very rough estimation under ideal conditions (e.g., without light absorption by intermediates), this LL dimer contribution might be observable by the TG method. Hence, we suggest another reason of the negligible LL dimer contribution in the TG signal; i.e., photo-reverse reaction from the K-intermediate to the dark state by the excitation light. Indeed, this type of the photo-reverse reaction has been reported for other rhodopsins.[5] Furthermore, the photo-reverse reaction of *T*aHeR was previously used for measurements of FTIR at low temperatures.[3] Therefore, these two factors (low reaction quantum yield and photo-reverse reaction from intermediates) could be the origins of the missing LL dimer contribution in the TG signal.

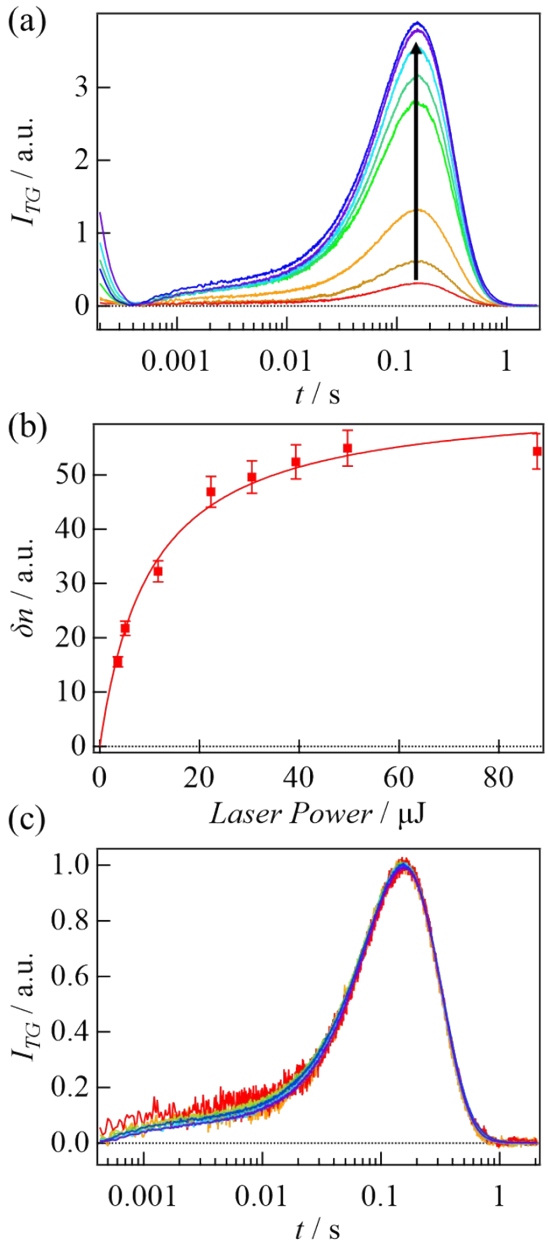

Figure S3. (a) The diffusion signals of *T*aHeR obtained under various excitation light intensities in the range of 3.6–88 μJ/pulse (the arrow indicates the order of pulse intensity from lowest to highest). (b) The square root of the TG signal intensity is plotted against pulse intensity. (c) The diffusion signals in Fig. S3(a) are normalized by the peak intensities.

SI-4. Probe light dependence of the O decay of *T*aHeR WT

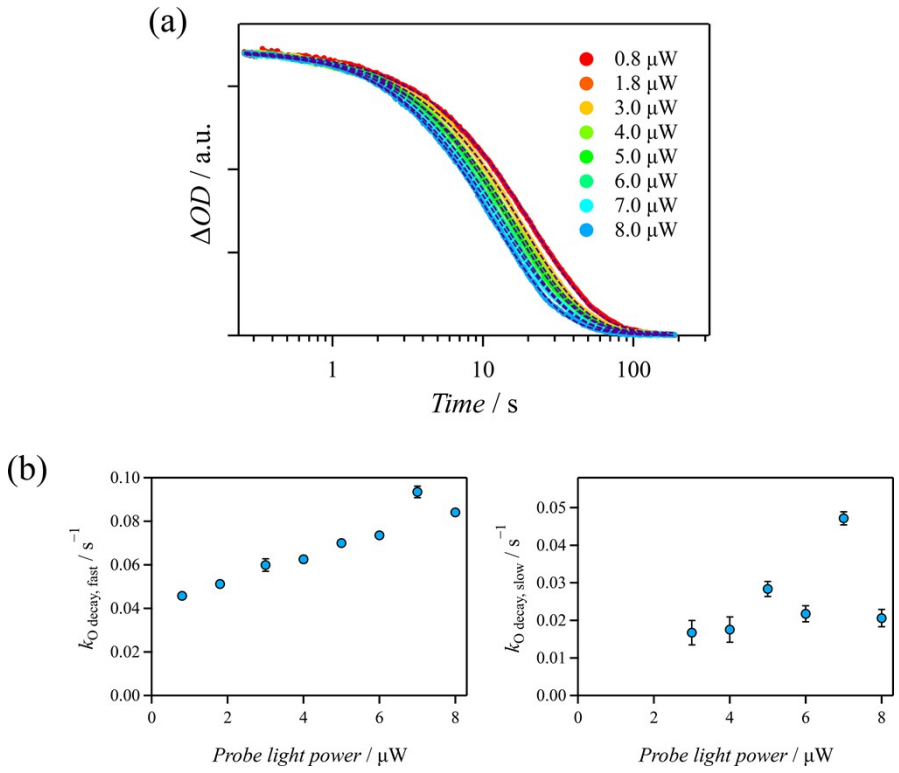

Figure S4. (a) Recovery curves of the O state after pulsed photoexcitation at 532 nm probed at 605 nm with different probe beam powers. The signal was normalized at the beginning of the decay. Dashed lines indicate the fitting lines by a multi-exponential function. (b) The rate constants of the fast (left) and slow (right) components of the bi-exponential O decay. While the major fast component was linearly accelerated by increasing the probe light power, the minor slow component appeared at the probe light power > 3 μW and its rate less depended on the probe light power.

SI-5. Time development of diffusion signal of Y93G.

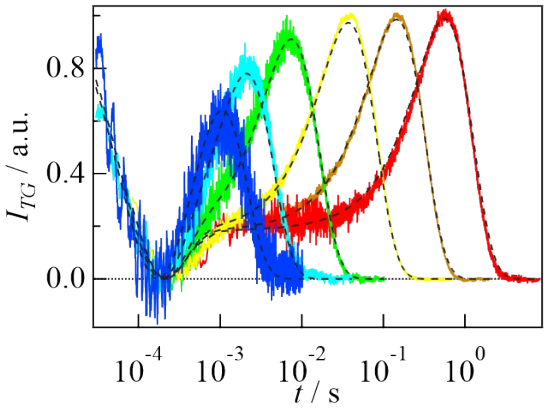

Figure S5. Granting wavenumber dependence on the molecular diffusion signal of Y93G. q^2 are 180, 85, 23, 4.4, 1.1, 0.29×10^{11} m^{-2} from left to right. The black broken lines are the best-fitted curves based on eq. (3).

Table S1. Sequences of primers used for mutagenesis of *T*aHeR Y93G.

Sense primer	Anti-sense primer
GCGACCGTGCTGTACTATCGTGG	TTCTTCAGGTTTTGAACGCCACG
CGTTCAAAACCTGAAGAA	ATAGTACAGCACGGTCGC

Supporting Information References

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