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SUPPLEMENTARY INFORMATION

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High-speed atomic force microscopy reveals dynamic molecular processes in photo-activated bacteriorhodopsin

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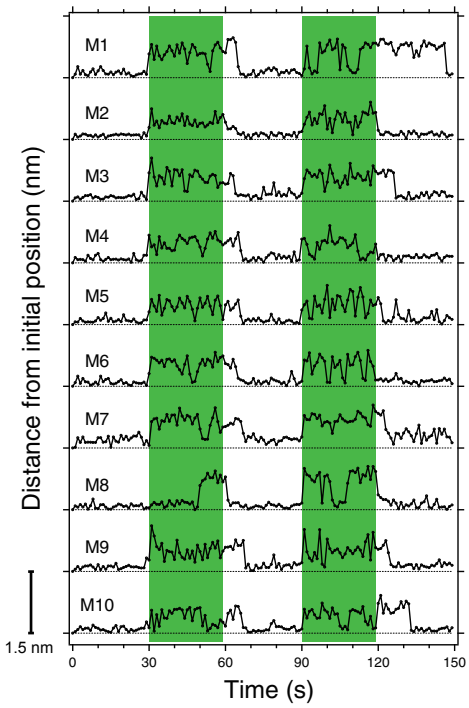
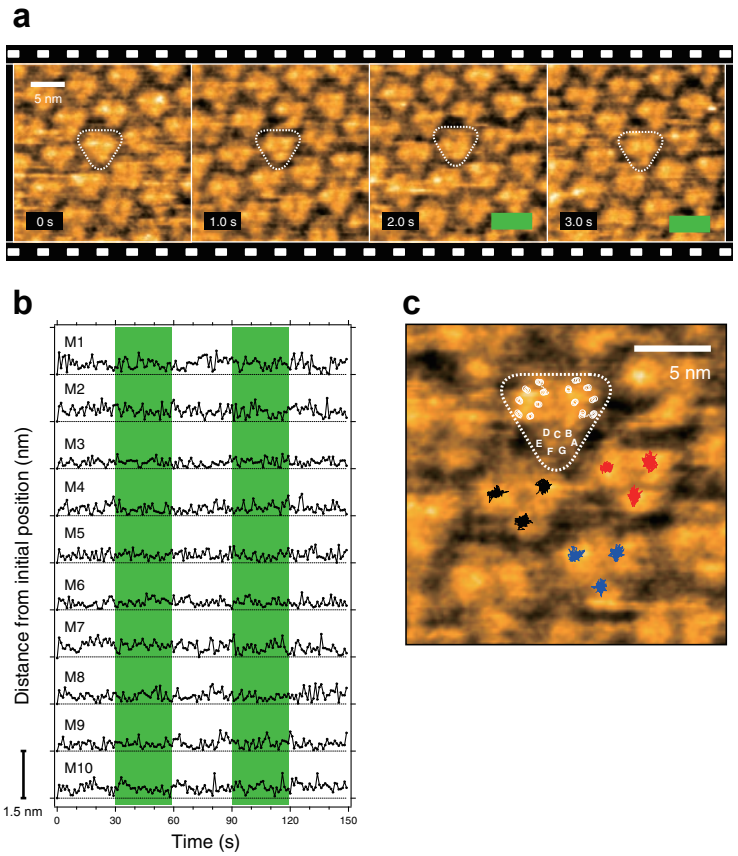


Supplementary Figure 1 | Displacement of the mass-center position from its initial position plotted as a function of time for 10 D96N bR monomers at the cytoplasmic surface (Supplementary Movie 1). The green regions indicate the illumination periods. The average displacement of the mass-center position is 0.69 ± 0.15 nm. Although the individual molecules show different lifetimes of the activated state, the initial bR recovers within 1 s. In addition, even under illumination (within green regions), the mass-center position often moves back to the initial position; some monomers repeat the photo-induced conformational changes under illumination (especially M5 and M6).



Supplementary Figure 2 | High-speed AFM images of the extracellular surface of D96N under dark or illuminated conditions. a, Successive AFM images of D96N bR adsorbed onto a mica surface in 10 mM Tris-HCl (pH 7) and 300 mM KCl. Frame rate: 1 fps; pixel size: 200×200 pixels (Supplementary Movie 5). A bR trimer is highlighted by the white triangles. The first two frames were captured in the dark condition, whereas the last two frames were captured under illumination of 532-nm green light with 0.5 μW (the green bars indicate illumination). **b**, Displacement of the mass-center position from its initial position plotted as a function of time for 10 bR monomers (Supplementary Movie 5). The green regions indicate the illumination periods. **c**, Enlarged AFM image of D96N at the extracellular surface in the unphotolyzed state. A bR trimer is highlighted by the white triangle, and the position of the α-helical extracellular ends (indicated by A–G) is derived from an atomic model of the unphotolyzed state. Traces of the mass-centers are superimposed on the AFM image (red, blue, and black marks).

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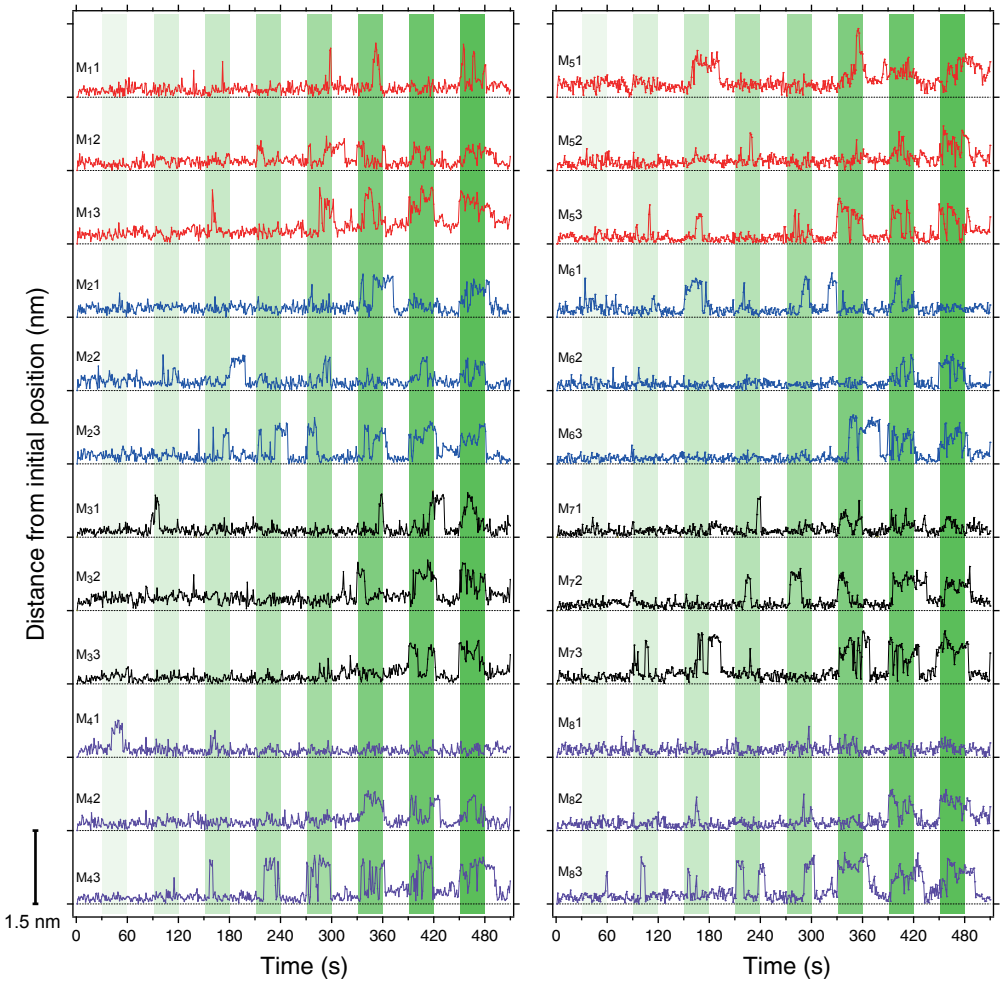

Supplementary Figure 3 | Displacement of mass-center position for D96N at the cytoplasmic surface under different light intensities. The figures show displacement of the mass-center position as a function of time measured for 24 bR monomers within eight different trefoils under different light intensities at pH 7 (Supplementary Movie 4). Here, "trefoil" means a triad of nearest neighbor bR molecules, each of which belongs to a different adjacent trimer. M_n1-M_n3 (n=1-8) indicate monomers within the n-th trefoil. During imaging, the light intensity was gradually increased in eight steps. The green regions correspond to the illumination periods, and their shade level indicates the relative intensity of the illumination. With increasing light intensity, the frequency of conformational changes is increased except for M_41 and M_81 monomers. In contrast, M_23, M_43, M_61, M_73, and M_83 monomers change their conformations even under weaker illumination.

Supplementary Movie 1 | High-speed AFM movie of the cytoplasmic surface of D96N at pH 7 during dark-illumination cycles. The purple membranes containing the D96N bR mutant were adsorbed onto a mica surface in 10 mM Tris-HCl (pH 7) and 300 mM KCl. Frame rate: 1 fps; pixel size: 150×150 pixels. The green bars shown in the AFM movie indicate green-light illumination. A bR trimer is highlighted by the white triangle at the first frame. This movie is played at ×10 speed.

Supplementary Movie 2 | High-speed AFM movie of the cytoplasmic surface of WT at pH 7 during dark-illumination cycles. The purple membranes containing WT bR were adsorbed onto a mica surface in 10 mM Tris-HCl (pH 7) and 300 mM KCl. Frame rate: 1 fps; pixel size: 150×150 pixels. The green bars shown in the AFM movie indicate light illumination. A bR trimer is highlighted by the white triangle at the first frame. This movie is played at ×10 speed.

Supplementary Movie 3 | High-speed AFM movie of the cytoplasmic surface of WT at pH 10 during dark-illumination cycles. The purple membranes containing WT bR were adsorbed onto a mica surface in 10 mM Tris-HCl (pH 10) and 300 mM KCl. Frame rate: 1 fps; pixel size: 150×150 pixels. The green bar shown in the AFM movie indicates light illumination. A bR trimer is highlighted by the white triangle at the first frame. This movie is played at ×10 speed.

Supplementary Movie 4 | High-speed AFM movie of the cytoplasmic surface of D96N at pH 7 with increasing light intensity. The purple membranes containing D96N bR were adsorbed onto a mica surface in 10 mM Tris-HCl (pH 7) and 300 mM KCl. Frame rate: 1 fps; pixel size: 200×200 pixels. While imaging, the light intensity was gradually increased in eight steps. The green bars indicate the illumination periods,

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and their shade level indicates the relative light intensity. A bR trimer is highlighted by the white triangle at the first frame. This movie is played at ×10 speed.

Supplementary Movie 5 | High-speed AFM movie of the extracellular surface of D96N at pH 7 during dark-illumination cycles. The purple membranes containing D96N bR were adsorbed onto a mica surface in 10 mM Tris-HCl (pH 7) and 300 mM KCl. Frame rate: 1 fps; pixel size: 200×200 pixels. The green bars shown in the AFM movie indicate light illumination. A bR trimer is highlighted by the white triangle at the first frame. This movie is played at ×10 speed.