Plasmid Construction and Site-directed Mutagenesis—The wild-type construct FP120 encoded a fusion protein in which full-length SRII and the 120 N-terminal residues of HtrII containing an additional His_6 tag at the C terminus are joined by the flexible linker ASASNGASA. The fusion gene was placed in plasmid pET21d (Novagen) under control of the T7 promoter. The plasmid was transformed into the *Escherichia coli* BL21 (DE3) strain. Single amino acid substitutions were performed using QuikChange II site-directed mutagenesis kit (Stratagene). The mutagenesis primer sequences were reported earlier (Bergo, V.B., Spudich, E. N., Rothschild, K. J. and Spudich, J. L. (2005) *J. Biol. Chem.* **280,** 28365 – 28369).

Protein Expression and Purification—The cells were grown in LB medium + ampicillin, 50 µg/ml, to an absorbance at 600 nm of ~ 0.4, and the protein synthesis was induced by addition of 1 mM isopropyl β-D-thiogalactopyranoside and 5 µM all-*trans*-retinal. After the induction period, the cells were centrifuged at 1000 x *g*, resuspended in 50 mM Tris/HCl, pH 7.0, 5 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride buffer and disrupted by a microfluidizer (Microfluidics Corp., Newton, MA), and the membranes were then harvested by ultracentrifugation. The membranes were solubilized in 300 mM NaCl, 10 mM imidazole, 50 mM potassium phosphate, pH 7.6, 1.5% octyl glucoside. After centrifugation of the solubilized membranes, the supernatant was incubated with nickel-nitrilotriacetic acid-agarose (Qiagen), and the His-tagged protein was eluted with a gradient of imidazole in 50 mM potassium phosphate, pH 7.6, 300 mM NaCl, 0.8% octyl glucoside in a Biologic Duoflow system (Bio-Rad).

Proteoliposome Reconstitution—Purified proteins were reconstituted in phospholipids by the dialysis procedure previously described (Krebs, M. P., Spudich, E. N., and Spudich, J. L. (1995) *Protein Expression Purif.* **6,** 780-788). In the work reported here the protein-to-lipid ratio was 1:6.5 (w/w). Extraction of halobacterial phospholipids used in the reconstitution procedures was performed as described previously (Bergo, V., Spudich, E. N., Scott, K. L., Spudich, J. L., and Rothschild, K. J. (2000) *Biochemistry* **39,** 2823-2830).

Rapid Scan Time-resolved FTIR Difference Spectroscopy—A stock suspension of membranes (10 mg/ml) containing SRII or the SRII-HtrII complex in 25 mM Tris-HCl, 50 mM NaCl, pH 8.0 buffer was kept in the refrigerator prior to use. The protein films were prepared by depositing 5–10 µl of this suspension onto a polished 2-mm-thick, 25-mm-diameter BaF_2 window (Wilmad, Buena, NJ) and then placing the sample in a dry box for ~ 1 h. The films were rehydrated via the vapor phase and then sealed in a temperature-controlled infrared cell (model TFC; Harrick Scientific Corp., Ossining, NY) using a second BaF_2 window. Alternatively, the samples were saturated with water by gently washing dry films with ~ 100 µl of the buffer solution and then removing the excess solution. The level of hydration was monitored by the absolute absorption IR spectra. The amount of water in samples with complete water saturation was at least 50% higher compared with the samples hydrated via the vapor phase as estimated from the increase of the absorption bands near 3400 and 1640 cm^{-1} because of the bulk water. For $H_2O \rightarrow H_2{}^{18}O$ exchange, the dry protein films were rehydrated with 50 µl of $H_2{}^{18}O$, allowed to dry and then rehydrated in the infrared cell as described above with $H_2{}^{18}O$. The

spectra were recorded with a BRUKER IFS 66 v/s FTIR spectrometer (Bruker Optics) as described previously (Bergo, V., Spudich, E. N., Spudich, J. L., and Rothschild, K. J. (2002) *Photochem. Photobiol.* **76,** 341–349) except that a 4000 cm^{-1} low-pass interference filter (OCLI, Santa Rosa, CA) was used instead of 2000 cm^{-1} and the spectra were recorded at a 8 cm^{-1} spectral resolution instead of 4 cm^{-1}. Four individual time slices collected within 50 ms after initiation of the photocycle were averaged to produce the final spectrum.