

Photoreaction Pathways of Bacteriorhodopsin and Its D96N Mutant as Revealed by in Situ Photoirradiation Solid-State NMR

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Table S1. ¹³C and ¹⁵N chemical shift values (ppm) of individual states of [20-¹³C, 14-¹³C]-retinal and [ε-¹⁵N]Lys²¹⁶-bacteriorhodopsin(BR) and Y185F-BR reported in the literature.

Protein	State	20- ¹³ C	14- ¹³ C	[ε- ¹⁵ N]Lys ²¹⁶	Configuration	Reference
BR	AT(BR568)	13.3 ^a	122.0 ^a	143.5 ^a	all-trans (13-trans, 15-anti)	a
	CS(BR548)	22.0 ^a	110.5 ^a	150.6 ^a	13-cis, 15-syn	a
	K		118.2 ^d	157.2 ^b	13-cis, 15-anti	b
	L165	15.5 ^b		166.0 ^b	13-cis, 15-anti	b
	L174		123.3 ^b	173.8 ^b	13cis, 15-anti	b
	L181	24.0 ^b	116.2 ^b	181.2 ^b	13-cis, 15-anti	b
	L185	24.5 ^b	116.2 ^b	186 ^b	13-cis, 15-anti	b
	M ₀	21.5 ^b	124.5 ^b	317 ^b	13-cis, 15-anti	b
			125.0 ^c	296.4 ^c		c
			123.2 ^c			
	M _n		124.6 ^c	288.8 ^c	13-cis, 15-anti	c
	M			295.2 ^d	13-cis, 15-anti	d
	N		115.2 ^d	150.7 ^d	13-cis, 15-syn	d
Y185F	AT	13.2 ^e	123.1 ^e		all-trans	e
	CS	21.7 ^e	110.0 ^e		13-cis, 15-syn	e
	CS*	18.0 ^e	115.3 ^e		13-cis, 15-syn	e
	N	19.2 ^e	125.4 ^e		13-cis, 15-anti	e
	O	13.2 ^e	123.1 ^e		all-trans	e

a, b, c, d, e : literatures which report the chemical shift values. References are listed in the last page.

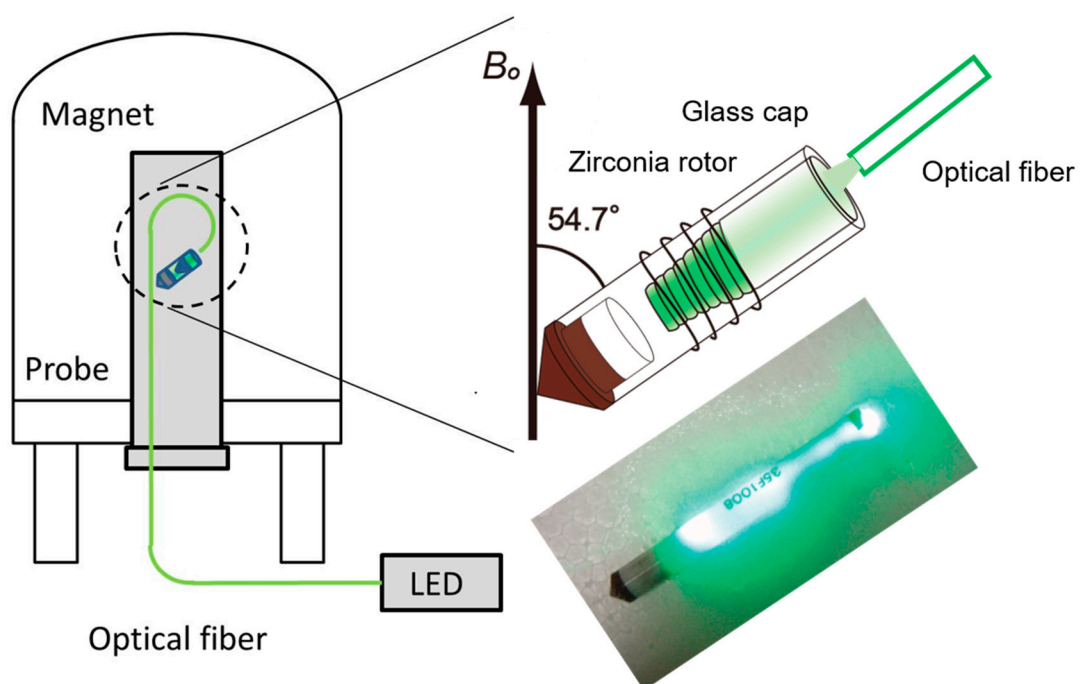


Figure S1. Schematic diagram of the *in situ* photoirradiation solid state NMR spectrometer. LED light sources (520 nm, 100 mW and 365 nm, 150 mW) are connected to optical fiber and guided from the bottom to the top part of the zirconia rotor in the probe head. The top part of the zirconia rotor is capped by a glass rod. Tip part of glass rod is ground to provide light illumination perpendicular to the rotor axis, so that light can be illuminated efficiently from inside the MAS rotor.

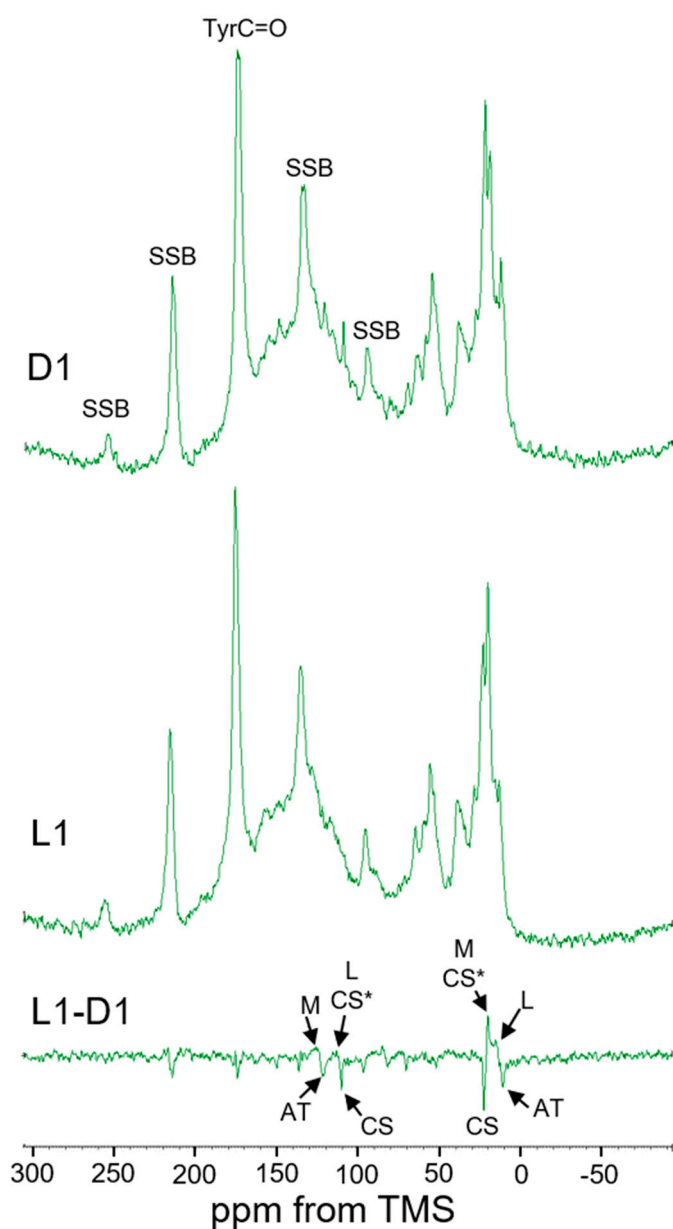


Figure S2. Full scale ^{13}C CP-MAS NMR spectra of $[\text{Tyr}^{13}\text{CO}]$ and $[20\text{-}^{13}\text{C}, 14\text{-}^{13}\text{C}]$ retinal-D96N-BR were recorded in the dark (D1; dark adapted state) and then under green light (L1) illumination at -30°C . The difference in spectra between L1 and D1 (L1-D1) indicated that the AT state changed to the M and L intermediates and the CS state changed to the CS^* intermediate. Line broadening factor of 60 Hz was applied. Scaling factor was not applied to get difference free induction decay signal between L1 and D1 that was Fourier transformed to get difference spectrum (L1-D1). $[\text{Tyr}^{13}\text{CO}]$ and $[20\text{-}^{13}\text{C}, 14\text{-}^{13}\text{C}]$ retinal-D96N-BR sample was used for light illumination experiments. However, only $[20\text{-}^{13}\text{C}, 14\text{-}^{13}\text{C}]$ retinal-D96N-BR sample is described in the manuscript because NMR signals of $[20\text{-}^{13}\text{C}, 14\text{-}^{13}\text{C}]$ retinal is mainly focussed on this study.

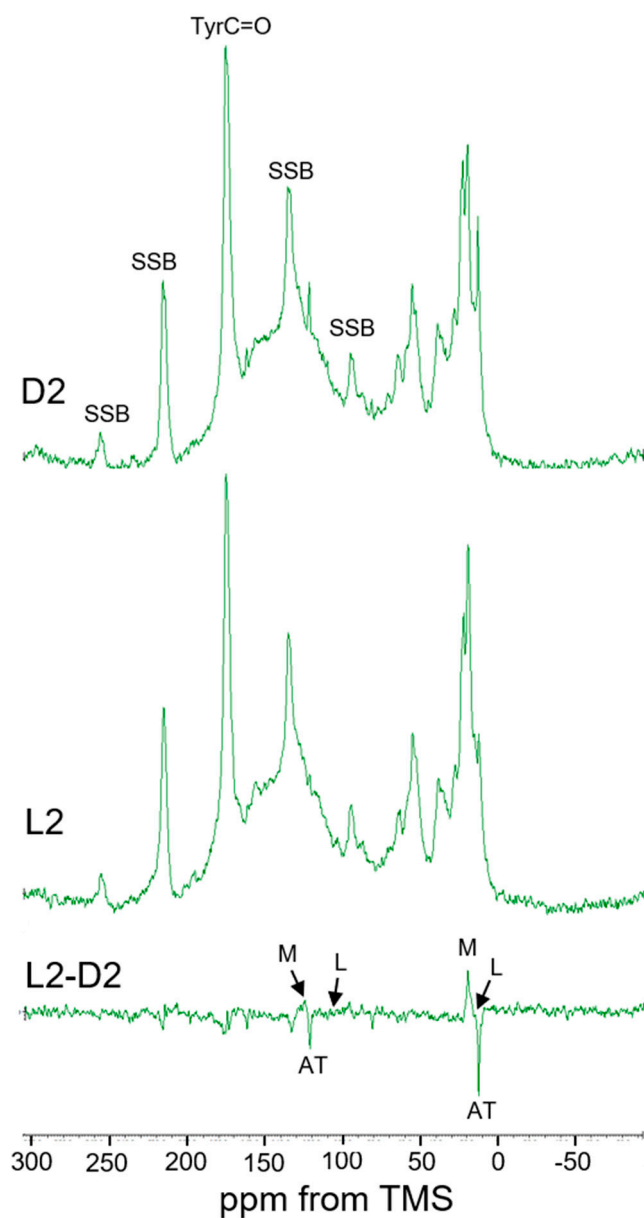


Figure S3. Full scale ^{13}C CP-MAS NMR spectra of $[\text{Tyr}^{13}\text{CO}]$ and $[20\text{-}^{13}\text{C}, 14\text{-}^{13}\text{C}]$ retinal-D96N-BR were recorded in the dark (D2; light adapted state) and then under green light (L2) illumination at -30°C . The difference in spectra between L2 and D2 (L2-D2) indicated that the AT state changed to the M and L intermediates. Line broadening factor of 60 Hz was applied. Scaling factor was not applied to get dephased free induction decay signal between L2 and D2 that was Fourier transformed to get difference spectrum (L2-D2). $[\text{Tyr}^{13}\text{CO}]$ and $[20\text{-}^{13}\text{C}, 14\text{-}^{13}\text{C}]$ retinal-D96N-BR sample was used for light illumination experiments. However, only $[20\text{-}^{13}\text{C}, 14\text{-}^{13}\text{C}]$ retinal-D96N-BR sample is described in the manuscript because NMR signals of $[20\text{-}^{13}\text{C}, 14\text{-}^{13}\text{C}]$ retinal is mainly focussed on this study.

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