Electronic Supplementary information (ESI) for

Photoisomerization of azobenzene units drives the photochemical reaction cycles of proteorhodopsin and bacteriorhodopsin analogues

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1. List of chromophores and proteins used in this study.

Table S1. List of chromophores and proteins used in this study.

Chromophores	**Proteins**				
Az I	PAz I	PAz I-D97N	PAz I-E108Q	PAz I-D227N	BAz I
Az II	PAz II	PAz II-D97N	PAz II-E108Q	PAz II-D227N	BAz II
Retinal	PR				BR

2. Photographs of POP expressed, and unexpressed *E. coli* cells incubated with azo chromophores Az I and Az II

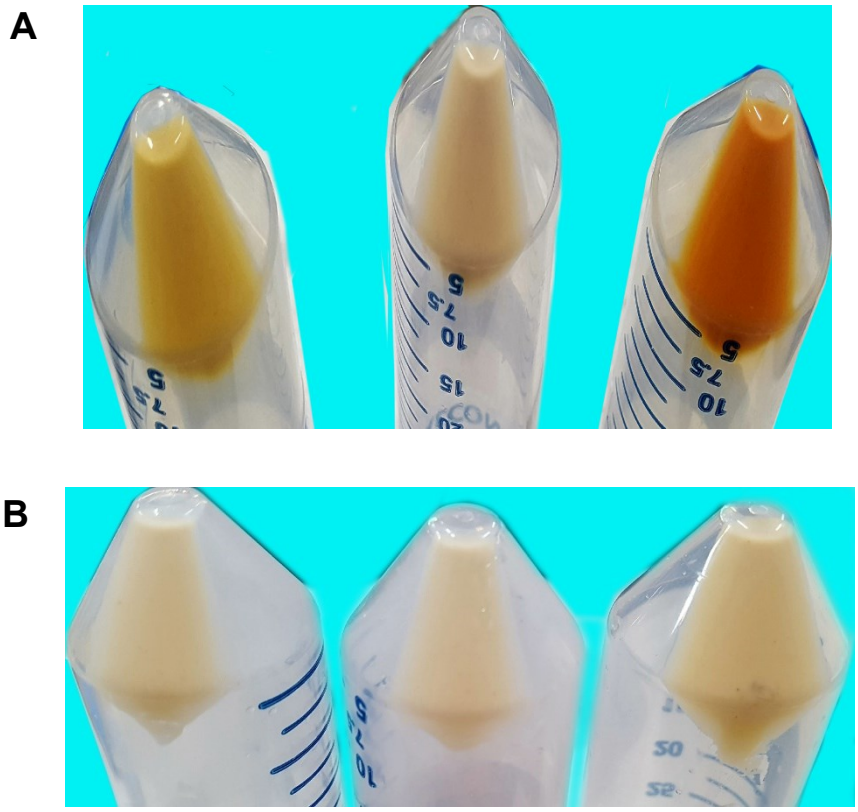


Fig. S1 A. Photographs of POP-expressed *E. coli* cells solids incubated with azo chromophore Az I (left) and Az II (right) and without azo chromophore (middle). **B.** Photograph of POP-unexpressed *E. coli* cells solids incubated with azo chromophores Az I (left) and Az II (right) and without azo chromophore (middle).

3. Flash-photolysis experimental and simulated curves of Az II and PAz II

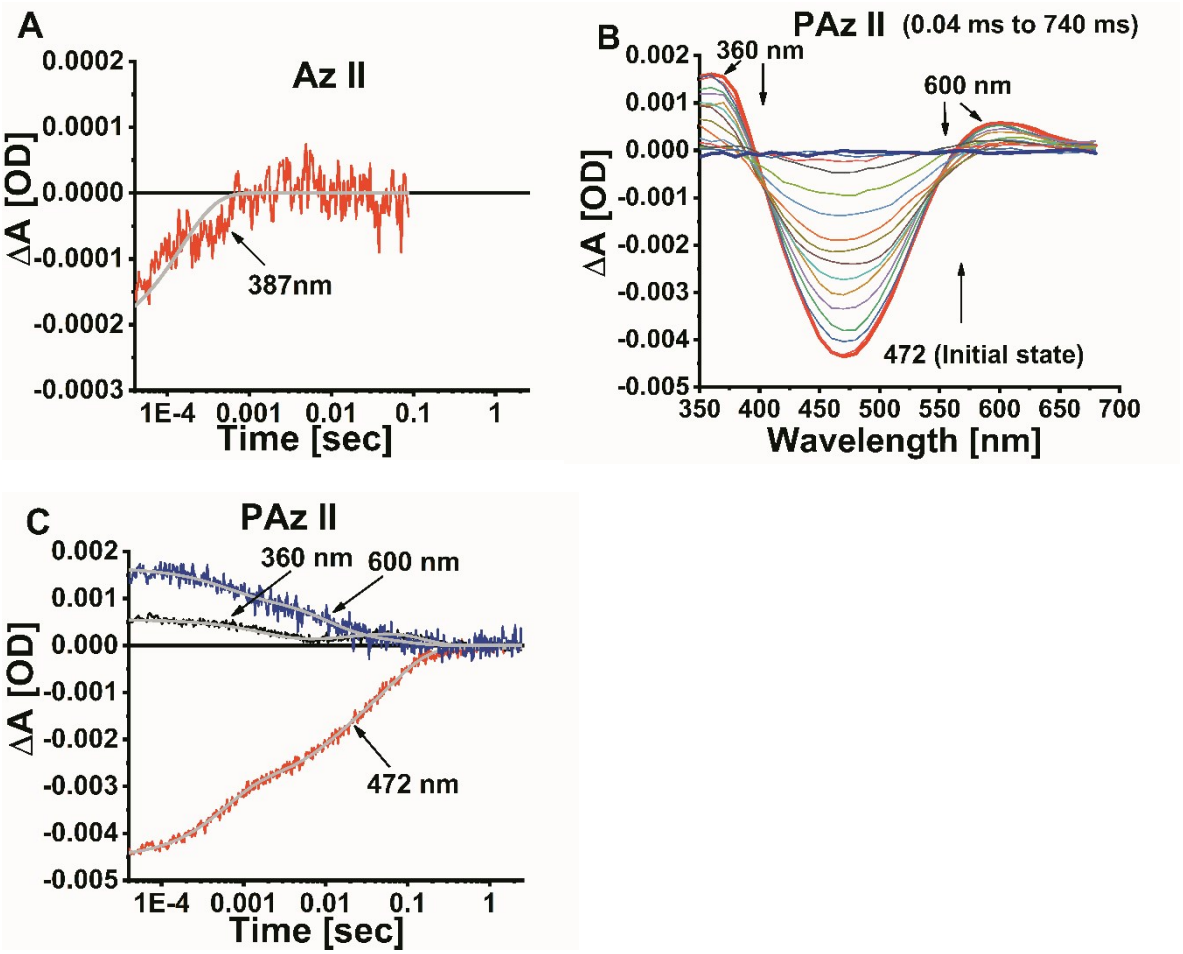


Fig. S2 A. Experimental (red) and simulated (light gray) flash-photolysis curves at λ_{max} of Az II. **B.** Flash-induced light-minus-dark difference absorption spectra of PAz II over the spectral range 350-700 nm and the time range of 0.04−740.0 ms. Herein red and black lines represent the spectra at 0.04 and 740.0 ms, respectively. The direction of the evolution of the spectral changes are indicated by arrows. **C.** Experimental and simulated flash photolysis curves at three characteristic wavelengths (360 nm, 472 nm and 600 nm) of PAz II. Fitting curves are shown as light gray lines.

4. UV-vis absorption spectra of Az II bound POP mutant proteins

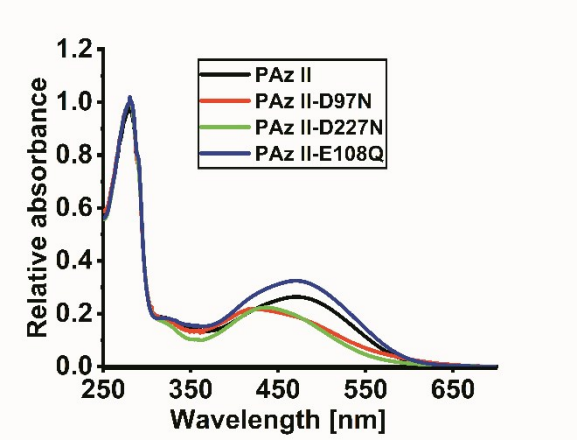


Fig. S3 UV-vis absorption spectra of PAz II (blank), PAz II-D97N (red), PAz II-D227N (green), and PAz II-E108Q (blue).

5. Photoinduced proton transfer reaction signals of Az II bound POP and POP mutant proteins

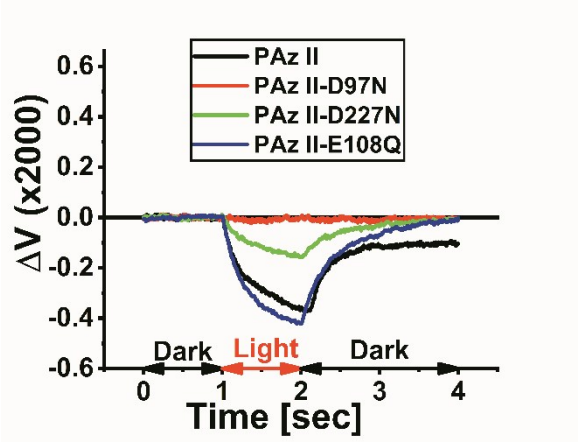


Fig. S4 Change in the signals for photoinduced proton transfer reaction of PAz II (blank), PAz II-D97N (red), PAz II-D227N (green), and PAz II-E108Q (blue).

6. Flash photolysis curves of PAz II-D97N, PAz II-D227N and PAz II-E108Q at different wavelengths

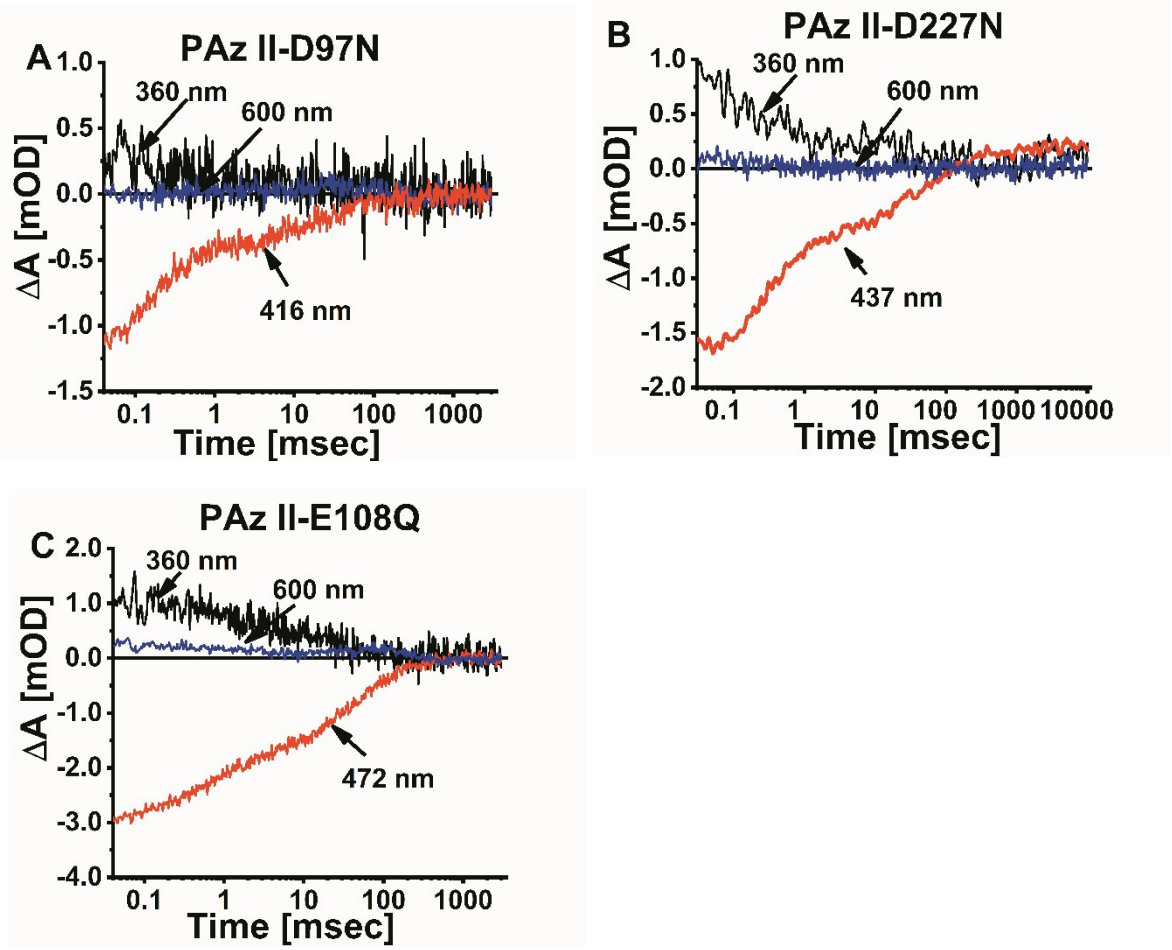


Fig. S5 Flash-induced absorbance changes at three characteristics wavelengths of **(A)** PAz II-D97N, **(B)** PAz II-D227N, and **(C)** PAz II-E108Q.

7. Flash photolysis experimental and simulated curves of BAz II at different wavelengths

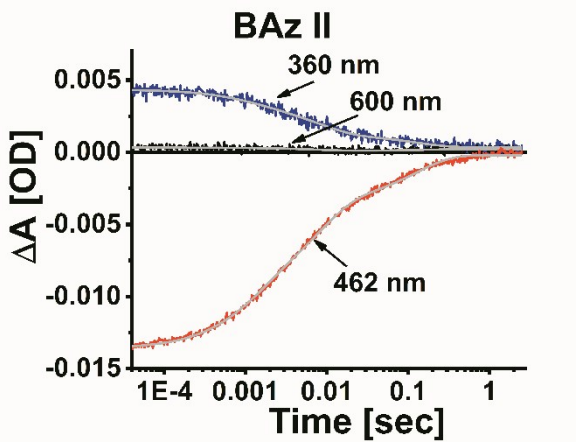


Fig. S6 Experimental and simulated flash photolysis curves at three wavelengths (360, 462, and 600 nm) of BAz II. Fitting curves are provided in light gray.

8. Fitting parameters of flash photolysis data of PAz II

Table S2 Fitting parameters of the flash photolysis data of PAz II recorded at 472, 360, and 600 nm.

Wave-length (nm)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	ϵP_1 (M^{-1} cm^{-1})	ϵP_2 (M^{-1} cm^{-1})	ϵP_3 (M^{-1} cm^{-1})	ϵP_0 (M^{-1} cm^{-1})	RSS
472	$1.77 \times 10^3 \pm 62.22$	102.0 ± 11.24	14.72 ± 0.59	130.8 ± 44.77	$7.69 \times 10^3 \pm 121.96$	$1.33 \times 10^4 \pm 367.50$	$2.12 \times 10^4 \pm 37.14$	3.12×10^{-6}
360	1.77×10^3	102.0	14.72	$7.78 \times 10^3 \pm 93.92$	$4.88 \times 10^3 \pm 94.51$	$1.18 \times 10^3 \pm 116.97$	14.40 ± 6.37	1.05×10^{-6}
600	1.77×10^3	102.0	14.72	$2.96 \times 10^3 \pm 50.77$	$1.25 \times 10^3 \pm 51.08$	991.0 ± 63.23	220.1 ± 32.75	3.04×10^{-6}

9. Fitting parameters of flash-photolysis data of BAz II

Table S3 Fitting parameters of the flash photolysis data of BAz II recorded at 462, 360, and 600 nm.

Wave-length (nm)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	ϵP_1 (M^{-1} cm^{-1})	ϵP_2 (M^{-1} cm^{-1})	ϵP_3 (M^{-1} cm^{-1})	ϵP_0 (M^{-1} cm^{-1})	RSS
462	879.3 ± 40.41	127.1 ± 5.01	8.01 ± 0.30	614.5 ± 28.15	8.39×10^3 ± 239.56	1.74×10^4 ± 104.36	2.24×10^4 ± 32.48	1.59×10^{-6}
360	879.3	127.1	8.01	7.05×10^3 ± 33.98	5.06×10^3 ± 64.57	1.93×10^3 ± 53.26	549.3 ± 37.23	1.59×10^{-6}
600	879.3	127.1	8.01	509.5 ± 21.91	478.8 ± 41.64	132.4 ± 34.34	334.3 ± 24.00	1.31×10^{-6}

10. NMR Spectral data

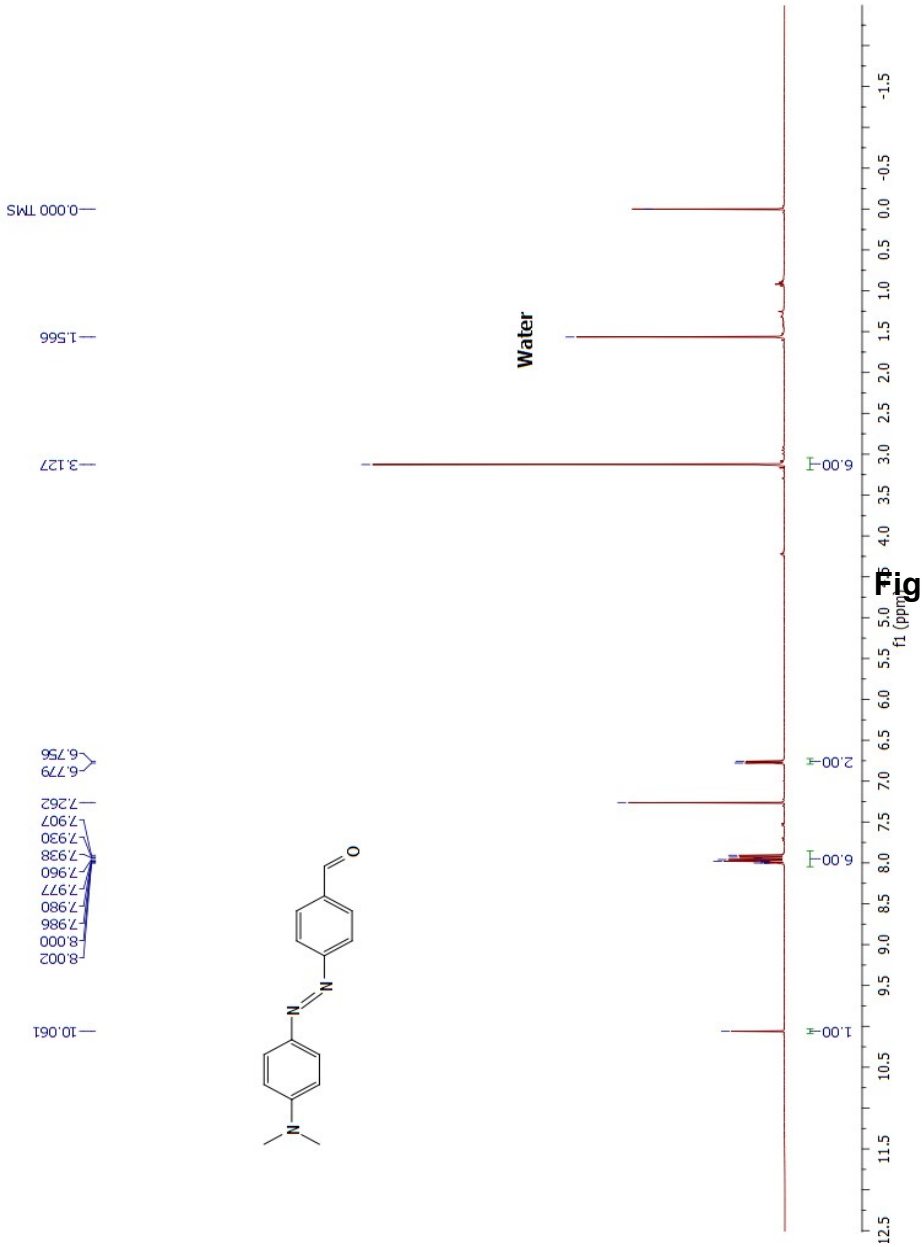


Fig. S7 ^{1}H NMR of Az I

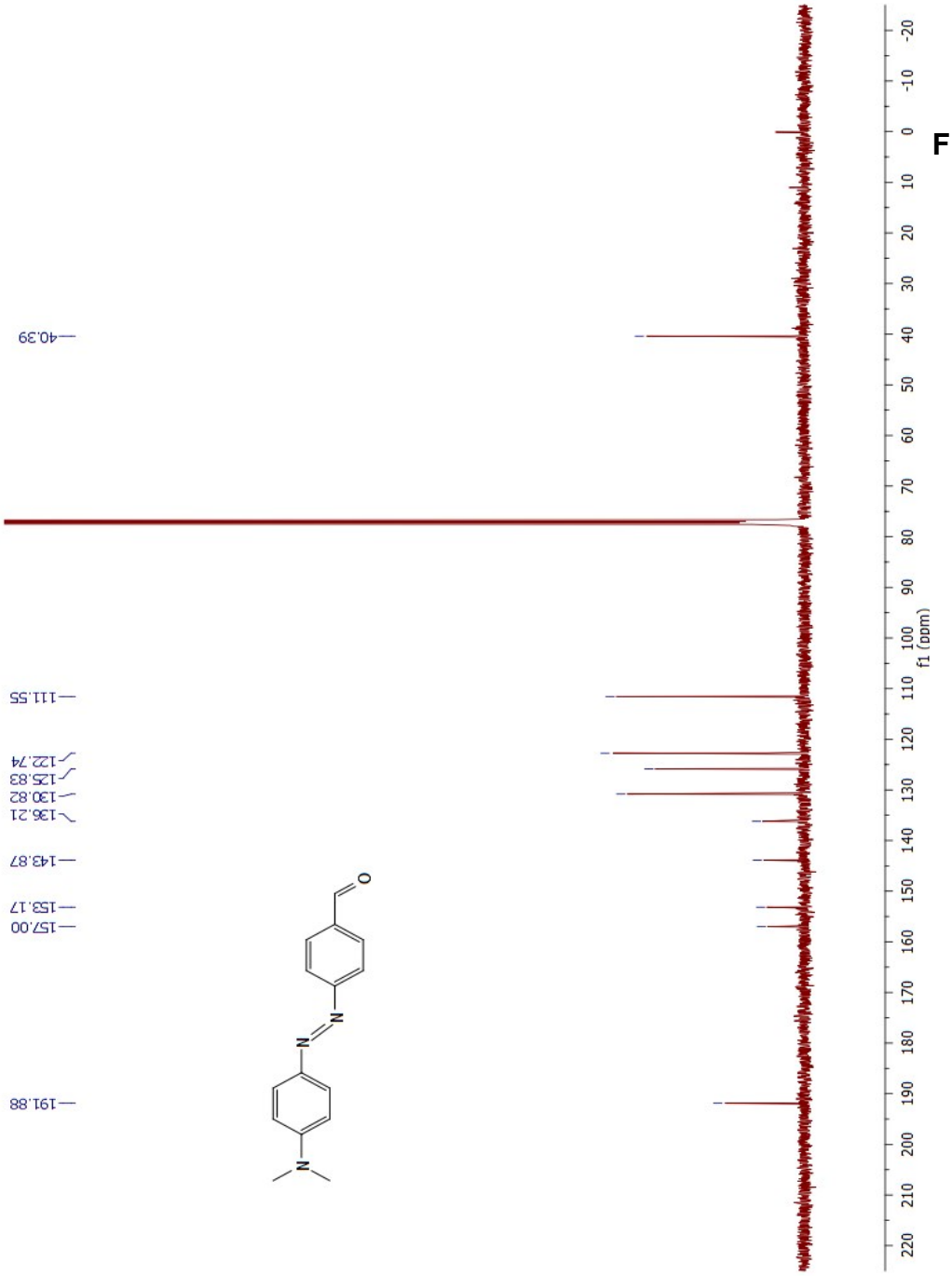
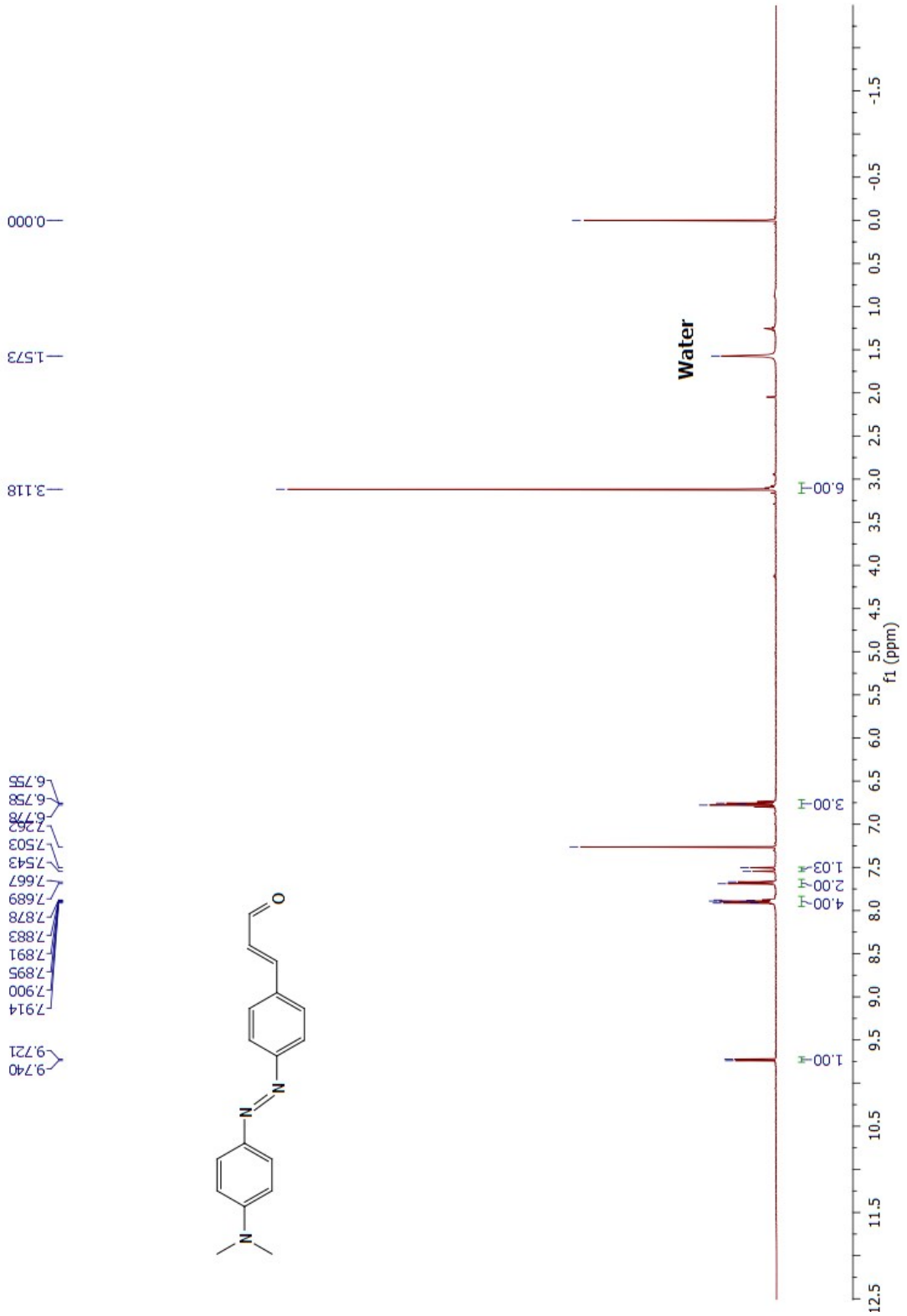


Fig. S8 ^{13}C NMR of Az I



. **S9** ^{1}H NMR of Az II

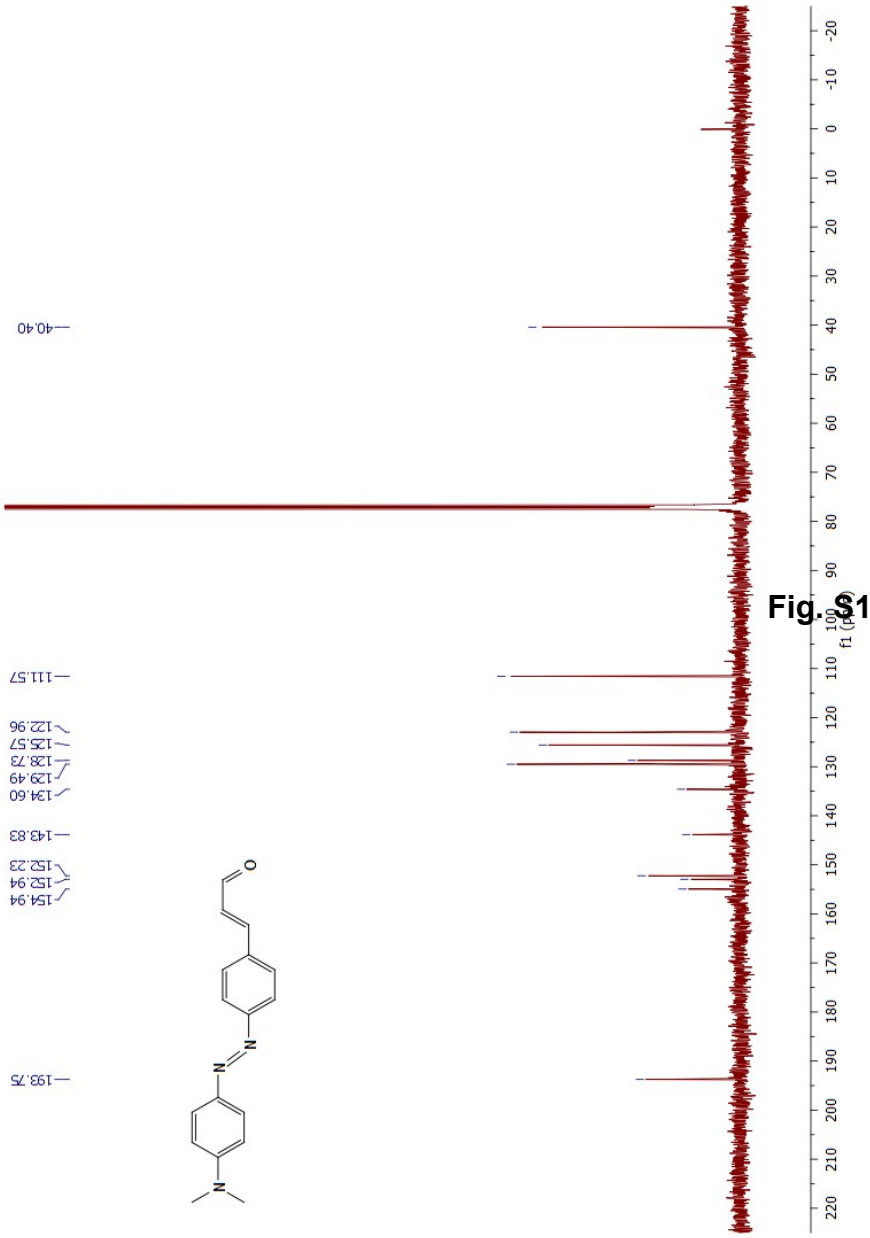


Fig. S10 ^{13}C NMR of Az II

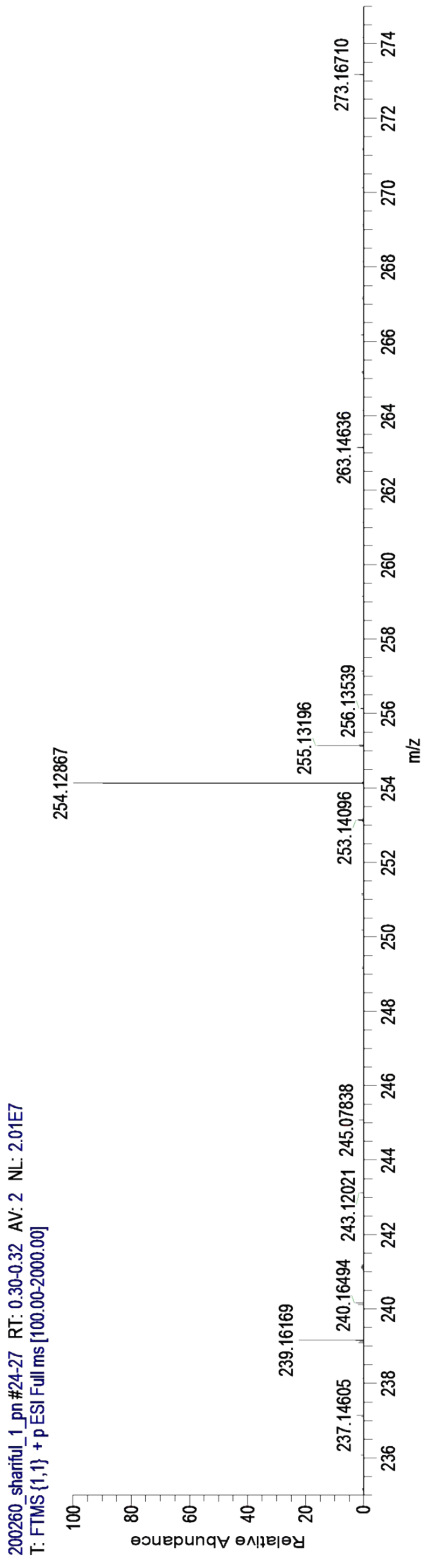


Fig. S11 High-resolution mass spectra of Az I. Observed and theoretical MS for $C_{15}H_{16}N_3O$ $[M + H]^+$: 254.12867 and 254.12879.

11. High-resolution mass spectra data

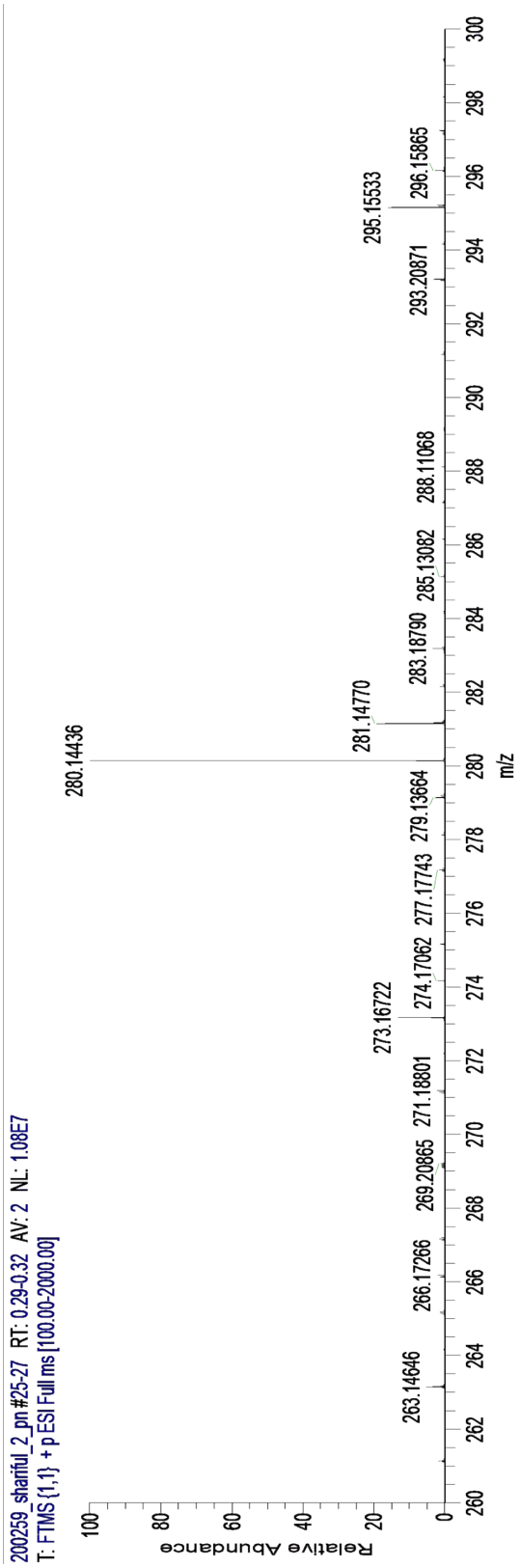


Fig. S12 High-resolution mass spectra of Az II. Observed and theoretical MS for $C_{17}H_{18}N_3O$ $[M + H]^+$: 280.14436 and 280.14444.

12. SDS-PAGE analysis of proteins

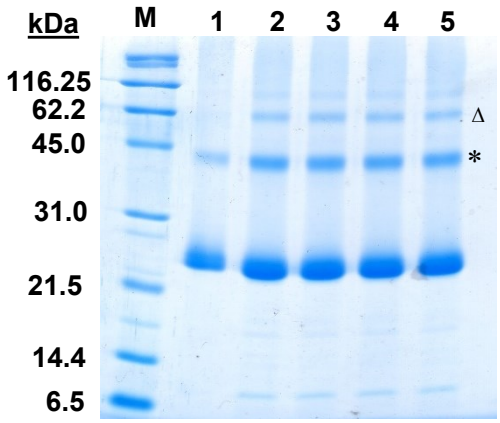


Fig. S13 SDS-PAGE analysis of azo chromophore Az I analogues of wild-type POP and POP mutant proteins. Lane M, protein molecular weight maker. Lanes 1-5 gave bands for wild-type PR, PAz I, PAz I-D97N, PAz I-D227N and PAz I-E108Q respectively. *,Δ SDS-PAGE bands might be due to the formation of dimer and trimer of POP.

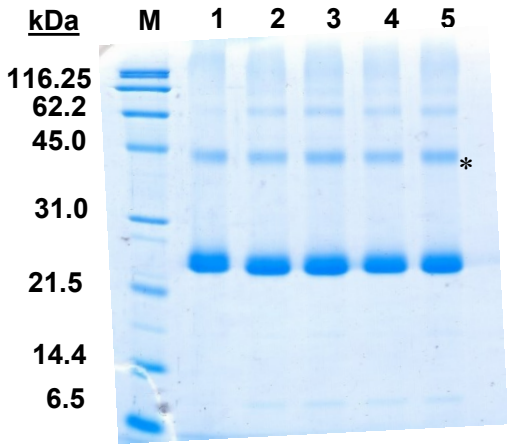


Fig. S14 SDS-PAGE analysis of azo chromophore Az II analogues of wild-type POP and POP mutant proteins. Lane M, protein molecular weight maker. Lanes 1-5 gave band for wild-type PR, PAz II, PAz II-D97N, PAz II-D227N and PAz II-E108Q respectively. * SDS-PAGE bands might be due to the formation of dimer of POP.

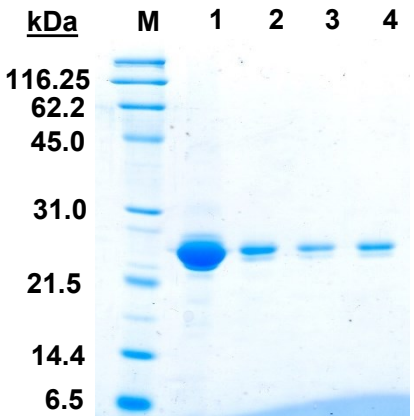


Fig. S15 SDS-PAGE analysis of azo analogues of BR. Lane M, protein molecular weight maker. Lanes 1-4 gave bands for wild-type BR, BOP, BAz I and BAz II respectively.