



# Supplementary Materials: Absorption and Emission Spectroscopic Investigation of the Thermal Dynamics of the Archaerhodopsin 3 Based Fluorescent Voltage Sensor QuasAr1

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## S1. Amino Acid Sequence of QuasAr1

The amino acid sequence of the here investigated mutated Arch protein QuasAr1 is displayed in Figure S1. The apoprotein molar mass is  $M_{apo} = 25.548$  kDa. It contains 11 phenylalanine (Phe, F), 7 tryptophan (Trp, W), and 12 tyrosine (Tyr, Y) residues.

```
      10      20      30      40      50      60
MDPIALQAGY DLLGDGRPET LWLGIGTLLM LIGTFYFLVR GWGVTDKDAR EYYAVTILVS

      70      80      90      100     110     120
GIASAAAYLSM FFGIGLTVES VGGEMLDIYY ARYAHWLFTT PLLLLHLALL AKVDRVTIGT

      130     140     150     160     170     180
LVGVDALMIV TGLIGALSHT AIARYSWWLF STICMIVVLY VLATSLRSAA KERGPEVAST

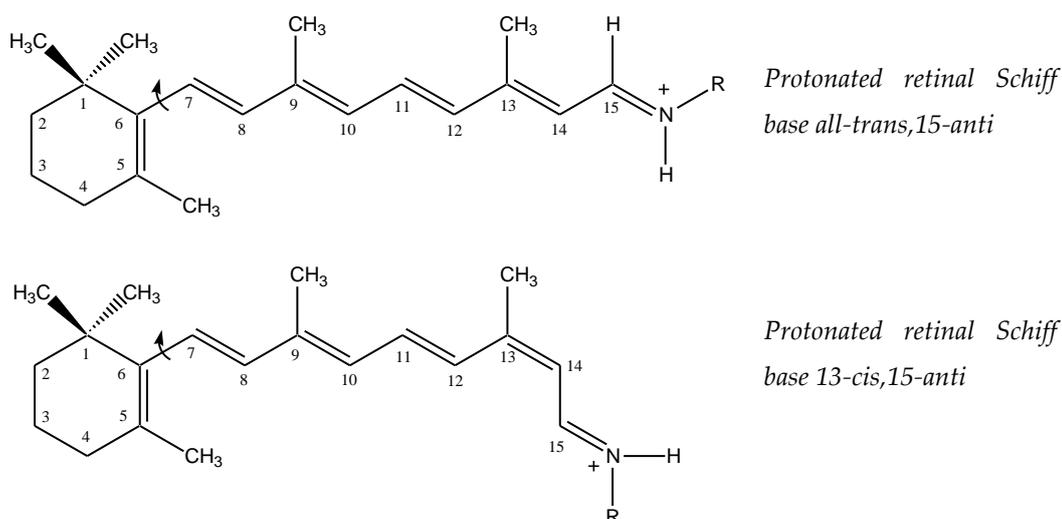
      190     200     210     220     230     240
FNTLTALVLV LWTAYPILWI IGTEGAGVVG LGIETLLFMV LDVTAKVGFV FILLRSRAIL

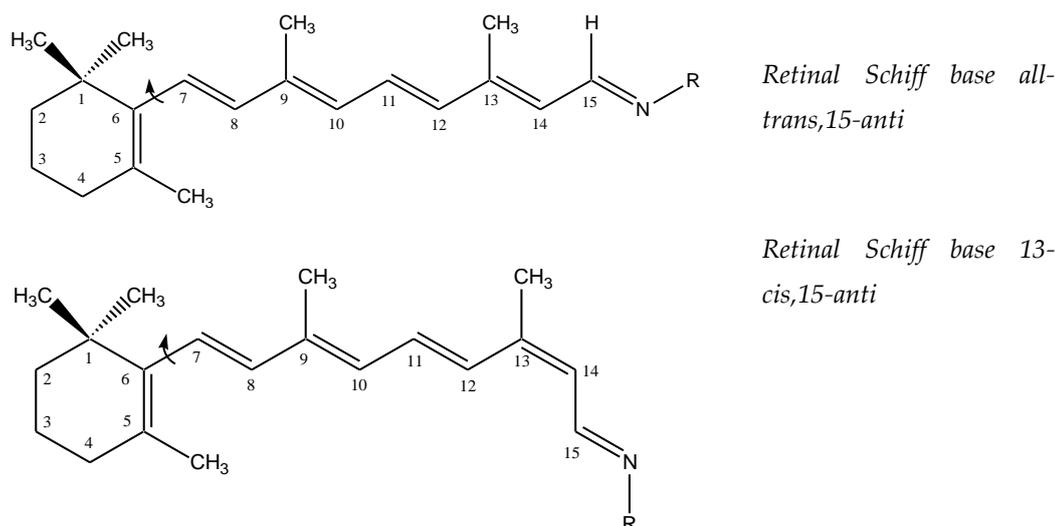
      250     260     270
GDTEAPEPSA GADENLYFQS LVDLEHHHHH H
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**Figure S1.** Amino acid sequence of QuasAr1 (Arch P60S, T80S, D95H, D106H, F161V).

## S2. Structural Formulae of Retinal Schiff Bases

In Figure S2 structural formulae of protonated retinal Schiff base (PRSB) isomers and unprotonated retinal Schiff base (RSB) isomers are shown.

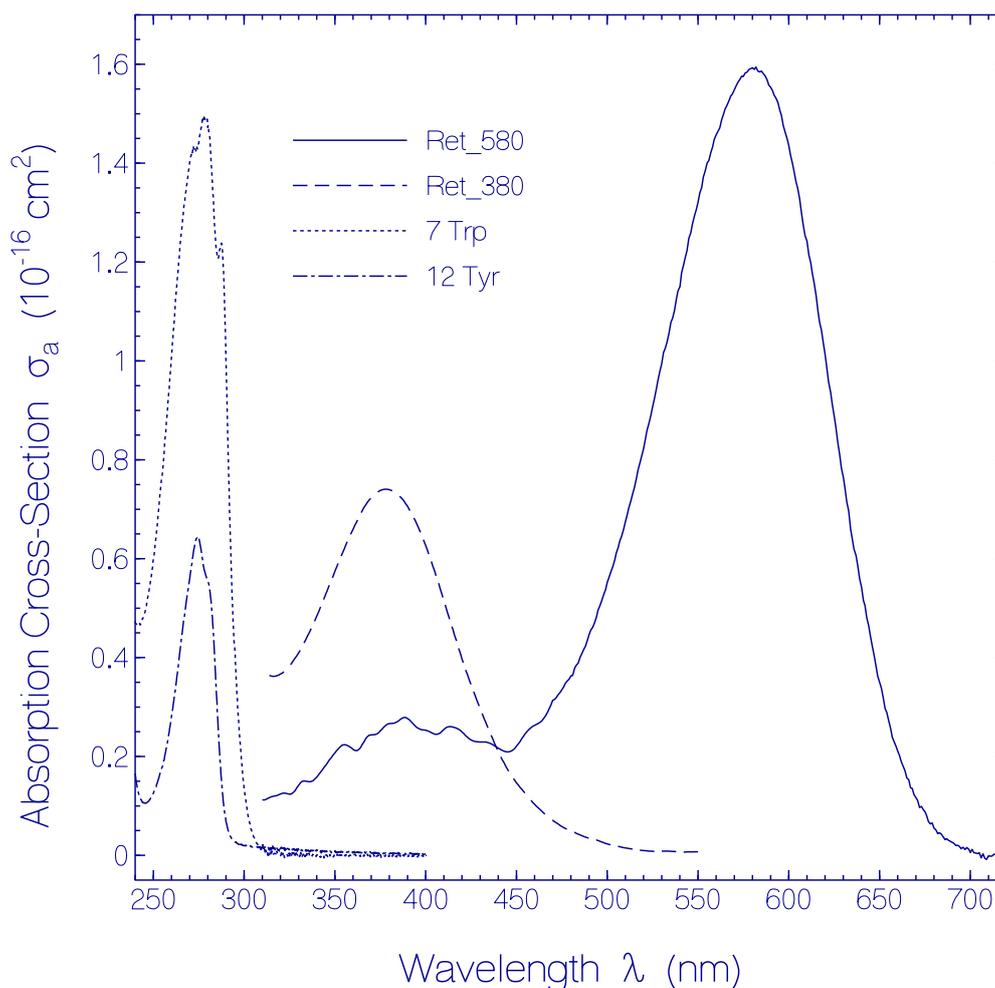




**Figure S2.** Structural formulae of some protonated retinal Schiff and neutral retinal Schiff base isomers.

### S3. Determination of Absorption Cross-section Spectra of Ret\_580 and Ret\_380

The molecule absorption cross-section spectra are derived from the molecule absorption coefficient spectra by the relation  $\sigma_a(\lambda) = \alpha_a(\lambda)/N$  where  $N$  is the molecule number density. The absorption coefficient  $\alpha_a(280 \text{ nm})$  of QuasAr1 of Figure 1 is composed of apoprotein and retinal contributions according to  $\alpha_a(280 \text{ nm}) = \alpha_{\text{apoprotein}}(280 \text{ nm}) + \alpha_{\text{retinal}}(280 \text{ nm}) = 3.73 \text{ cm}^{-1}$ . The retinal contribution is set to be  $\alpha_{\text{retinal}}(280 \text{ nm}) = 0.37 \text{ cm}^{-1}$  [51] giving  $\alpha_{\text{apoprotein}}(280 \text{ nm}) = 3.36 \text{ cm}^{-1}$ . The apoprotein absorption coefficient is given by  $\alpha_{\text{apoprotein}} = N_{\text{apoprotein}} \times \sigma_{\text{apoprotein}}(280 \text{ nm})$  where  $N_{\text{apoprotein}}$  is the apoprotein number density. The apoprotein absorption cross-section is composed of the contribution of 11 Phe ( $\sigma_{\text{Phe}}(280 \text{ nm}) = 3.8 \times 10^{-21} \text{ cm}^2$  [84]), 7 Trp ( $\sigma_{\text{Trp}}(280 \text{ nm}) = 2.10 \times 10^{-17} \text{ cm}^2$  [84]), and 12 Tyr ( $\sigma_{\text{Tyr}}(280 \text{ nm}) = 4.62 \times 10^{-18} \text{ cm}^2$  [84]) giving  $\sigma_{\text{apoprotein}}(280 \text{ nm}) = 2.025 \times 10^{-16} \text{ cm}^2$  and  $N_{\text{apoprotein}} = 1.66 \times 10^{16} \text{ cm}^{-3}$ . The retinal number density  $N_{\text{retinal}}$  is equal to the apoprotein number density (one retinal isomer is covalently bound to one opsin apoprotein) and is composed of the number density  $N_{\text{Ret}_580}$  of protonated retinal Schiff base Ret\_580 and the number density  $N_{\text{residual retinals}}$  of residual retinal components according to  $N_{\text{retinal}} = N_{\text{Ret}_580} + N_{\text{residual retinals}} = N_{\text{apoprotein}}$ . The ratio of the number densities  $N_{\text{residual retinals}}/N_{\text{Ret}_580}$  is approximated by the ratio of  $S_0$ - $S_1$  absorption coefficient integrals  $\int_{310 \text{ nm}}^{465 \text{ nm}} \alpha_{\text{a,residual retinals}}(\tilde{\nu})d\tilde{\nu} / \int_{440 \text{ nm}}^{700 \text{ nm}} \alpha_{\text{a,Ret}_580}(\tilde{\nu})d\tilde{\nu}$  ( $\tilde{\nu} = \lambda^{-1}$  is wavenumber) of Figure 1 giving a value of  $N_{\text{residual retinals}} = 0.16 \times N_{\text{Ret}_580} = (0.16/1.16) \times N_{\text{retinal}} = 2.3 \times 10^{15} \text{ cm}^{-3}$  and  $N_{\text{Ret}_580} = N_{\text{retinal}}/(1 + 0.16) = 1.43 \times 10^{16} \text{ cm}^{-3}$ . The resulting absorption cross-section spectrum  $\sigma_{\text{a,Ret}_580}(\lambda) = \alpha_{\text{a,Ret}_580}(\lambda)/N_{\text{Ret}_580}$  is displayed by the solid curve in Figure S3.



**Figure S3.** Absorption cross-section spectra. Solid curve: Ret\_580 (PRSB) of QuasAr1 in pH 8 Tris buffer (determined from Figure 1 and estimated Ret\_580 number density) Dashed curve: Ret\_380 (RSB, determined from heat degraded sample at 69.6 °C of Figure 4b). Dotted curve: absorption cross-section spectrum of 7 Trp molecules (taken from [84]). Dash-dotted curve: absorption cross-section spectrum of 12 Tyr molecules (taken from [84]).

The  $S_0$ – $S_1$  absorption cross-section integral of Ret\_580 is  $\sigma_{a,Ret_{580,int}} = \int_{440\text{ nm}}^{700\text{ nm}} \sigma_{a,Ret_{580}}(\tilde{\nu})d\tilde{\nu} = 6.1 \times 10^{-13}$  cm. Its oscillator strength is given by [85]

$$f_{Ret_{580}} = 1.1296 \times 10^{12} \frac{9n}{(n^2 + 2)^2} \sigma_{a,Ret_{580,int}} \quad (S1)$$

where  $n$  is the refractive index averaged over the absorption band (here  $n \approx n_{\text{water}} \approx 1.333$ ). Insertion of parameters gives  $f_{Ret_{580}} = 0.58$ . The full spectral half-width of the  $S_0$ – $S_1$  absorption band of Ret\_580 is  $\delta\tilde{\nu}_{Ret_{580}} = 3600$   $\text{cm}^{-1}$ .

The absorption cross-section spectrum of the deprotonated retinal Schiff base Ret\_380 is shown by the dashed curve in Figure S3. It was obtained from the absorption coefficient spectrum  $\alpha_a(\lambda, \vartheta=69.6$  °C) of Figure 4b and the assumption that practically all PRSB Ret\_580 is deprotonated to Ret\_380 so that the number density of Ret\_380 is now  $N_{Ret_{380}} \approx N_{\text{retinal}} \approx 1.66 \times 10^{16}$   $\text{cm}^{-3}$ . The  $S_0$ – $S_1$  absorption cross-section integral of Ret\_380 is  $\sigma_{a,Ret_{380,int}} \approx \int_{310\text{ nm}}^{550\text{ nm}} \sigma_{a,Ret_{380}}(\tilde{\nu})d\tilde{\nu} = 5.2 \times 10^{-13}$  cm. Its oscillator strength is

$$f_{Ret_{380}} = 1.1296 \times 10^{12} \frac{9n}{(n^2 + 2)^2} \sigma_{a,Ret_{380,int}} \quad (S2)$$

giving  $f_{\text{Ret}_380} \approx 0.49$  ( $n \approx n_{\text{water}} \approx 1.34$ ). The full spectral half-width of the  $S_0$ - $S_1$  absorption band of Ret\_380 is  $\delta\tilde{\nu}_{\text{Ret}_380} = 7200 \text{ cm}^{-1}$ .

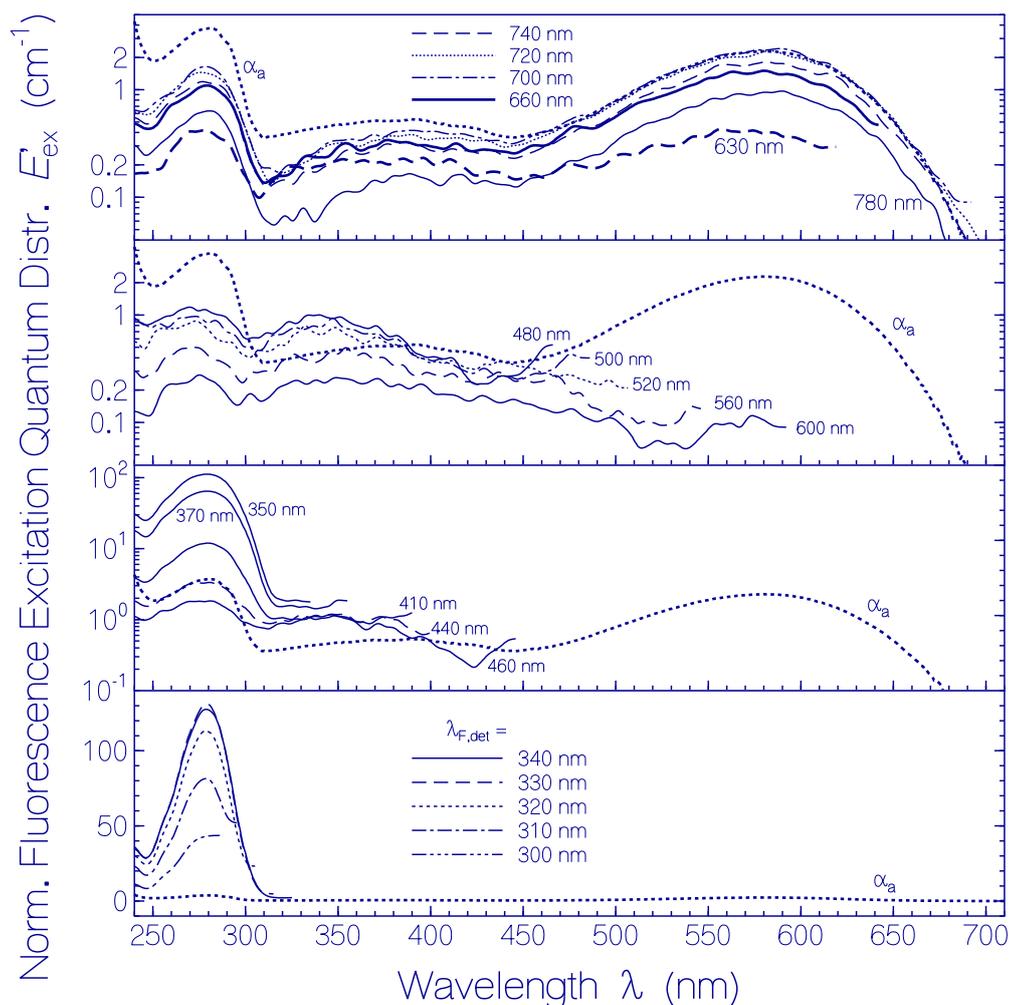
The  $S_0$ - $S_1$  absorption cross-section integral of tyrosine is  $\sigma_{\text{Tyr.int}} \approx \int_{243 \text{ nm}}^{300 \text{ nm}} \sigma_{\text{Tyr}}(\tilde{\nu})d\tilde{\nu} = 1.87 \times 10^{-14} \text{ cm}$ , and the oscillator strength of tyrosine is  $f_{\text{Tyr}} = 1.1296 \times 10^{12} (9n / (n^2 + 2)^2) \sigma_{\text{Tyr.int}} = 0.0175 (n_{\text{water}}(280 \text{ nm}) = 1.353)$ .

The  $S_0$ - $S_1$  absorption cross-section integral of tryptophan is  $\sigma_{\text{Trp.int}} \approx \int_{237 \text{ nm}}^{320 \text{ nm}} \sigma_{\text{Trp}}(\tilde{\nu})d\tilde{\nu} = 1.162 \times 10^{-13} \text{ cm}$ , and the oscillator strength of tryptophan is  $f_{\text{Trp}} = 1.1296 \times 10^{12} (9n / (n^2 + 2)^2) \sigma_{\text{Trp.int}} = 0.1089$ .

#### S4. Normalized Fluorescence Excitation Quantum Distributions of Fresh Thawed QuasAr1

In Figure S4 normalized fluorescence excitation quantum distributions  $E'_{\text{ex}}(\lambda) = E'_{\text{ex},\lambda_{\text{F,det}}}(\lambda)$  of a fresh thawed QuasAr1 sample in pH 8 Tris buffer are shown for fluorescence detection wavelengths  $\lambda_{\text{F,det}}$  in the range from 300 nm to 780 nm. The normalization is made so that  $E'_{\text{ex},\lambda_{\text{F,det}}=720 \text{ nm}}(\lambda = 582 \text{ nm})$  is equal to  $\alpha_a(\lambda = 582 \text{ nm})$ , i.e.  $E'_{\text{ex},\lambda_{\text{F,det}}}(\lambda) = E_{\text{ex},\lambda_{\text{F,det}}}(\lambda) \alpha_a(582 \text{ nm}) / E_{\text{ex},\lambda_{\text{F,det}}=720 \text{ nm}}(\lambda = 582 \text{ nm})$ .  $E_{\text{ex},\lambda_{\text{F,det}}}(\lambda)$  is given by  $E_{\text{ex},\lambda_{\text{F,det}}}(\lambda) = \gamma_\alpha S_{\lambda_{\text{F,det}}}(\lambda) / S_{\text{exc}}(\lambda)$  where  $S_{\lambda_{\text{F,det}}}(\lambda)$  is the detected fluorescence signal at  $\lambda_{\text{F,det}}$  as a function of the excitation wavelength  $\lambda$ ,  $S_{\text{exc}}(\lambda)$  is the excitation light spectrum, and  $\gamma_\alpha = [\alpha_a(\lambda)l + \alpha_a(\lambda_{\text{F,det}})d] / \{1 - \exp[-\alpha_a(\lambda)l - \alpha_a(\lambda_{\text{F,det}})d]\}$  is an absorption correction factor ( $l$  is the sample length along the excitation direction,  $d$  is the sample width along the fluorescence detection direction) [80,82].

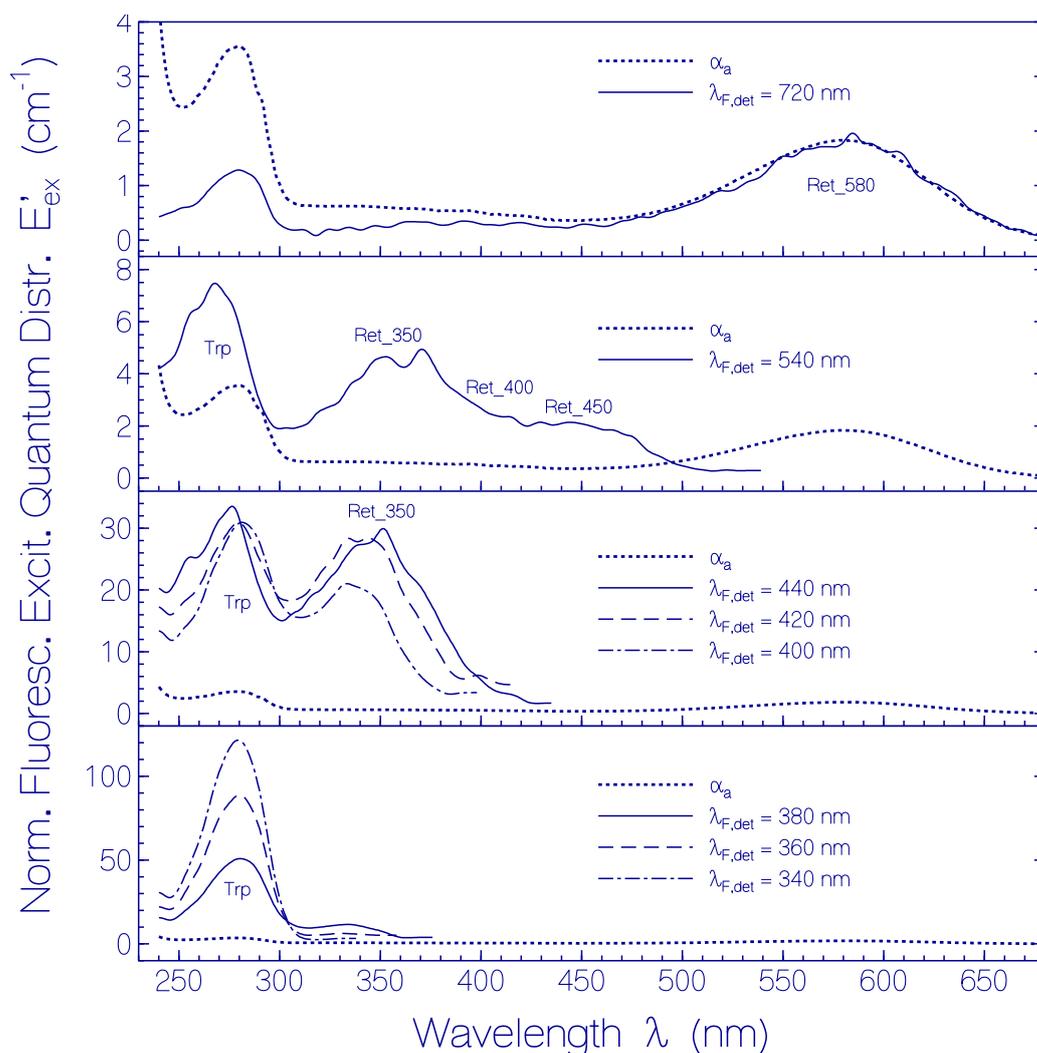
The curve  $E'_{\text{ex},\lambda_{\text{F,det}}=720 \text{ nm}}(\lambda)$  represents the absorption coefficient spectrum of Ret\_580. It was applied to determine  $\alpha_{\text{a,Ret}_580}(\lambda)$  in Figure 1 and it was used in Figure S3 to determine the absorption cross-section spectrum of Ret\_580. The normalized fluorescence excitation spectra for  $\lambda_{\text{F,det}} = 600 \text{ nm}$  to 410 nm indicate the presence of retinal isomers absorbing in the wavelength region from 500 nm to 310 nm. The normalized fluorescence excitation spectra for  $\lambda_{\text{F,det}} = 370 \text{ nm}$  to 300 nm indicate the QuasAr1 apoprotein absorption caused by Trp and Tyr.



**Figure S4.** Normalized fluorescence excitation quantum distributions  $E'_{ex, \lambda_{F, det}}(\lambda) = E_{ex, \lambda_{F, det}}(\lambda) \times \alpha_a(582 \text{ nm}) / E_{ex, \lambda_{F, det}=720 \text{ nm}}(582 \text{ nm})$  of fresh thawed QuasAr1 in pH 8 Tris buffer for the fluorescence detection wavelengths  $\lambda_{F, det}$  listed in the sub-figures. The thick dotted curves show the absorption coefficient spectrum  $\alpha_a(\lambda)$  of the investigated sample.

#### S5. Normalized Fluorescence Excitation Quantum Distributions of QuasAr1 Stored at 2.5 °C

Normalized fluorescence excitation quantum distributions  $E'_{ex}(\lambda) = E'_{ex, \lambda_{F, det}}(\lambda) = E_{ex, \lambda_{F, det}}(\lambda) \alpha_a(582 \text{ nm}) / E_{ex, \lambda_{F, det}=720 \text{ nm}}(\lambda = 582 \text{ nm})$  of QuasAr1 in pH 8 Tris buffer stored in the dark at 2.5 °C for 80 days are shown in Figure S5 for different fluorescence detection wavelengths  $\lambda_{F, det}$ . For comparison the absorption coefficient spectrum  $\alpha_a(\lambda)$  of the QuasAr1 sample stored in the dark for 80 days is included in the sub-figures.



**Figure S5.** Normalized fluorescence excitation quantum distributions  $E'_{ex,\lambda_{F,det}}(\lambda) = E_{ex,\lambda_{F,det}}(\lambda) \times \alpha_a(582\text{ nm}) / E_{ex,\lambda_{F,det}=720\text{ nm}}(582\text{ nm})$  of QuasAr1 in pH 8 Tris buffer stored in the dark at  $2.5 \pm 0.5$  °C over a period of 80 days. The fluorescence detection wavelengths  $\lambda_{F,det}$  are listed in the legends.

In the top sub-figure  $E'_{ex,\lambda_{F,det}=720\text{ nm}}(\lambda)$  resembles the absorption coefficient spectrum of Ret\_580 in the wavelength range  $\lambda \geq 310$  nm. For  $\lambda < 310$  nm  $E'_{ex,\lambda_{F,det}=720\text{ nm}}(\lambda)$  is dominated by apoprotein Tyr and Trp absorption and subsequent Förster-type energy transfer to Ret\_580.

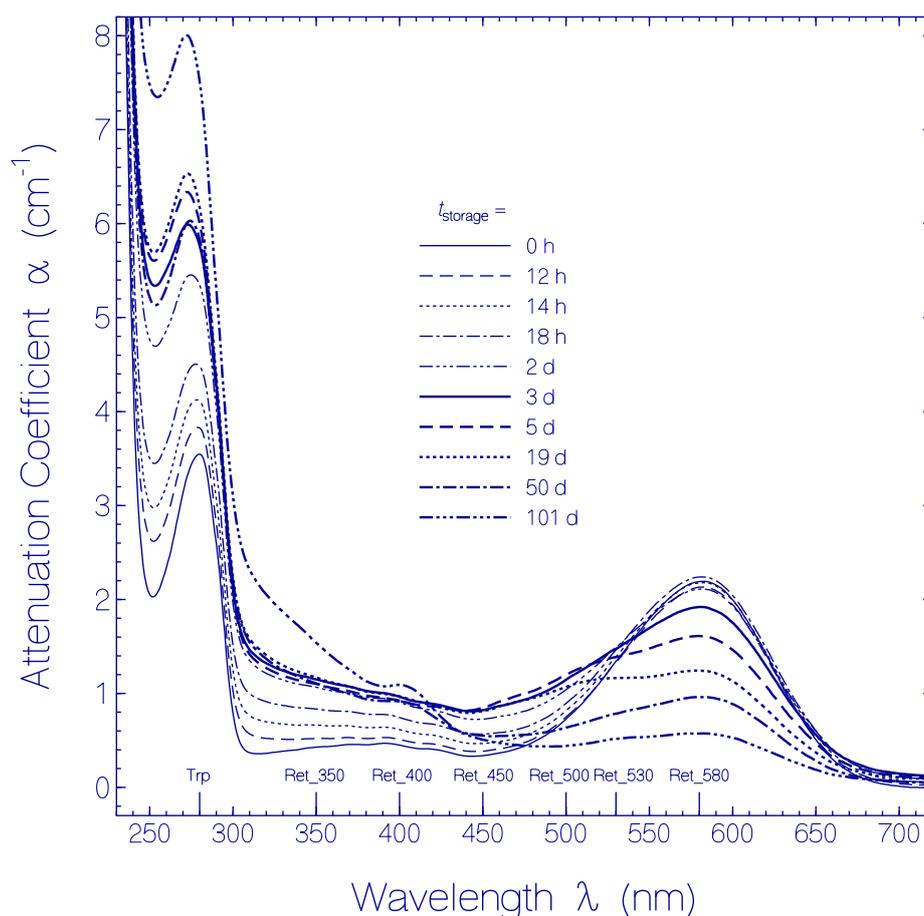
In the second-top sub-figure  $E'_{ex,\lambda_{F,det}=540\text{ nm}}(\lambda)$  is displayed. It is determined by fluorescence emission at 540 nm from Ret\_450, Ret\_400, and Ret\_350.  $E'_{ex,\lambda_{F,det}=540\text{ nm}}(\lambda < 310\text{ nm})$  is dominated by photo-excitation of apoprotein Trp and Tyr followed by Förster-type energy transfer to Ret\_350, Ret\_400, and Ret\_450.

In the second-lowest sub-figure normalized fluorescence quantum distributions are shown for  $\lambda_{F,det} = 440$  nm (contribution from Ret\_400 and Ret\_350), for  $\lambda_{F,det} = 420$  nm (contribution from Ret\_350), and for  $\lambda_{F,det} = 400$  nm (contribution from Ret\_350 and from Trp).  $E'_{ex,\lambda_{F,det}=440\text{ nm}, 420\text{ nm}, 400\text{ nm}}(\lambda < 310\text{ nm})$  is dominated by photo-excitation of apoprotein Trp and Tyr followed by Förster-type energy transfer to Ret\_350 and Ret\_400.

In the bottom sub-figure normalized fluorescence quantum distributions are shown for  $\lambda_{F,det} = 380$  nm (contribution from Ret\_350 and from Trp), for  $\lambda_{F,det} = 360$  nm (contribution from Ret\_350 and from Trp), and for  $\lambda_{F,det} = 340$  nm (contribution from Trp).

## S6. Temporal Development of Attenuation Coefficient Spectra of QuasAr1 Stored at Room Temperature

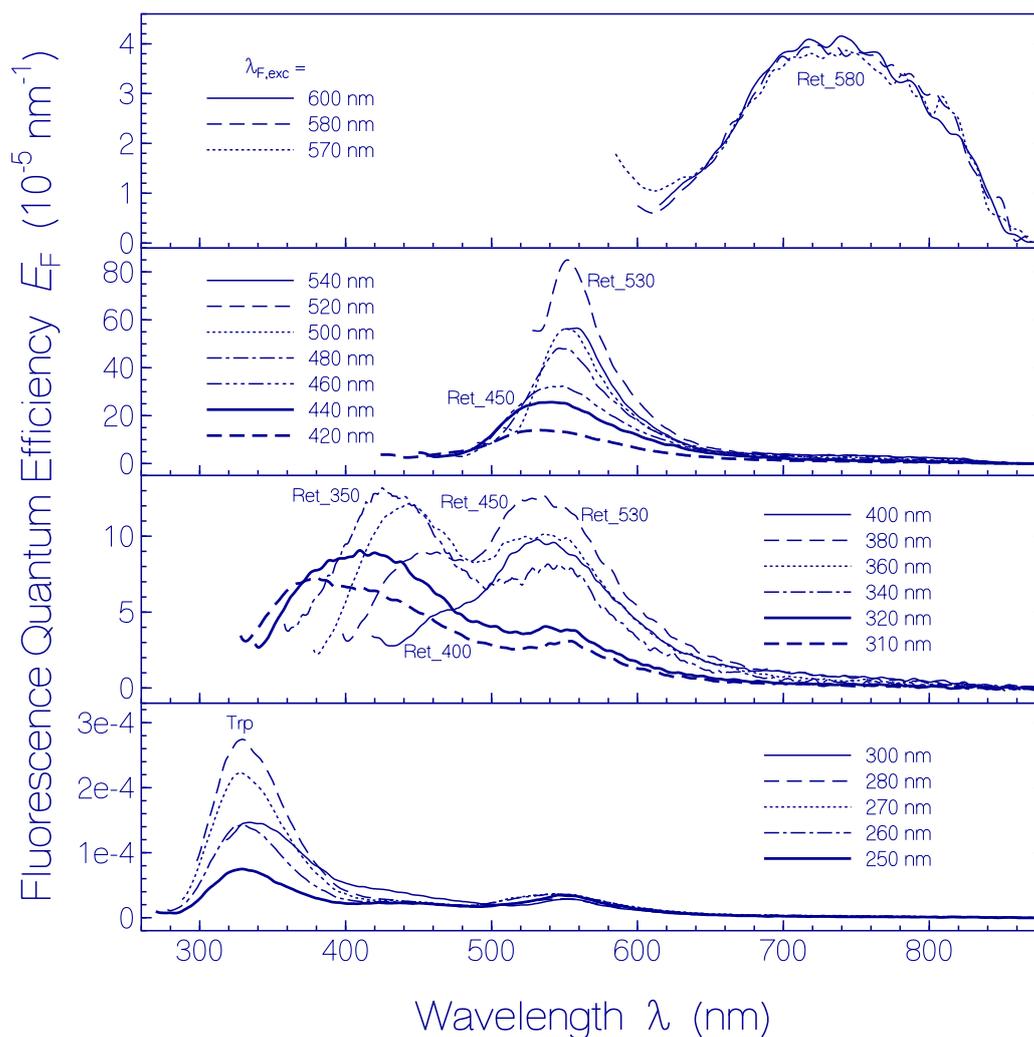
The temporal development of the attenuation coefficient spectra  $\alpha(\lambda)$  of QuasAr1 in pH 8 Tris buffer in the dark at room temperature is shown in Figure S6. The attenuation decreased around 580 nm (PRSB, Ret\_580), new attenuation built-up and decreased around 500 nm (PRSB, Ret\_500). The attenuation increased with storage time around 400 nm (RSB, Ret\_400) and around 350 nm (RSB, Ret\_350). The temporal increase of attenuation in the range from 320 nm to 250 nm is attributed to apoprotein restructuring with enlarged apoprotein absorption strength.



**Figure S6.** Temporal development of attenuation coefficient spectra  $\alpha(\lambda)$  of QuasAr1 in pH 8 Tris buffer at room temperature ( $\theta = 21\text{--}25\text{ }^{\circ}\text{C}$ ) in the dark. The storage times are listed in the legend.

## S7. Fluorescence Emission Spectra of QuasAr1 Stored at Room Temperature for 101 Days

In Figure S7 fluorescence quantum distributions of QuasAr1 in Tris 8 buffer stored for 101 days in the dark at room temperature are displayed. The curves are similar to curves shown in Figure 8 for sample storage of 50 days at room temperature in the dark. The main difference is the enhanced overall fluorescence quantum distribution in the second top part of Figure S7 since there the absorption contribution of Ret\_580 is reduced compared to the absorption contribution of Ret\_530 and Ret\_450 with higher specific fluorescence quantum yields.

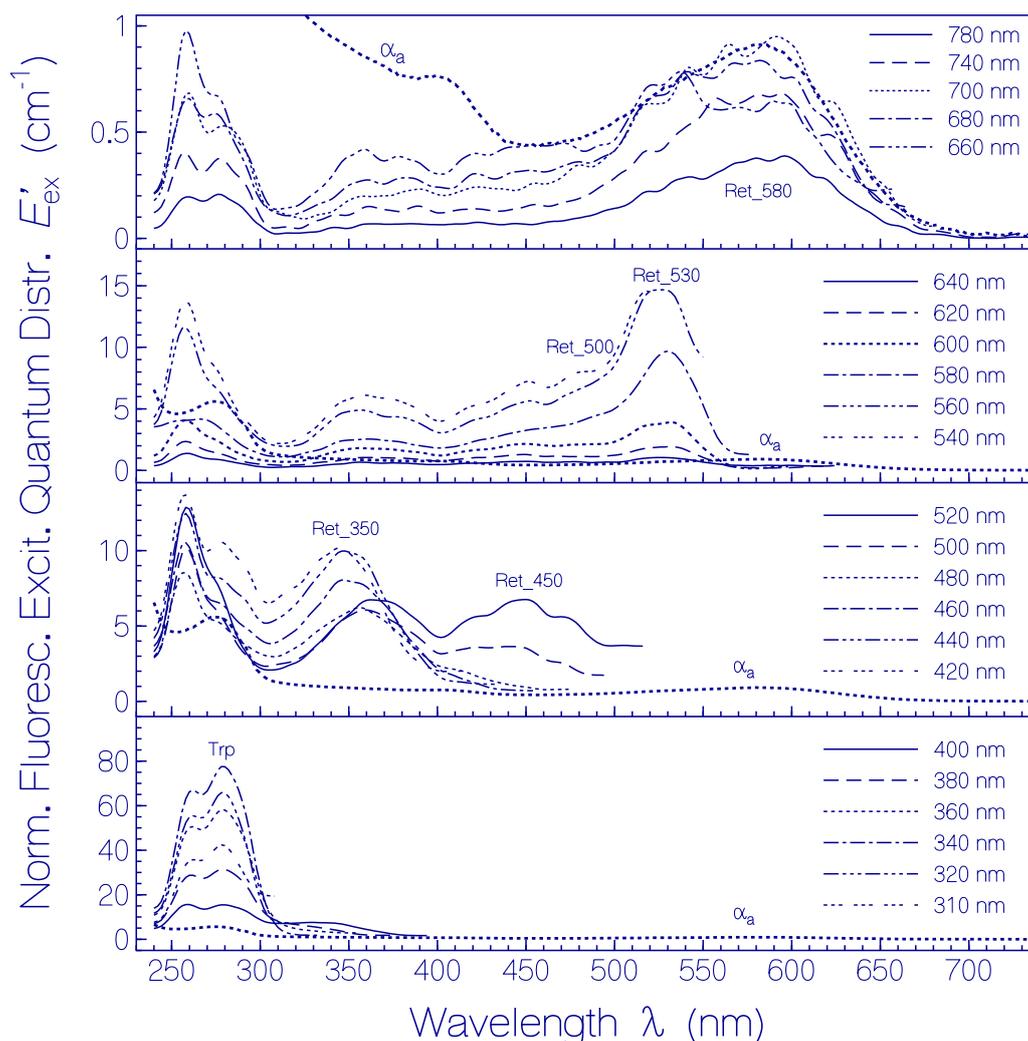


**Figure S7.** Fluorescence emission quantum distributions  $E_F(\lambda)$  of QuasAr1 in pH 8 Tris buffer stored at room temperature ( $\vartheta = 21\text{--}25\text{ }^\circ\text{C}$ ) for a duration of 101 days. The fluorescence excitation wavelengths  $\lambda_{F,exc}$  are listed in the legends.

### S8. Normalized Fluorescence Excitation Quantum Distributions of QuasAr1 Stored at Room Temperature

Normalized fluorescence excitation quantum distributions  $E'_{ex}(\lambda) = E'_{ex,\lambda_{F,det}}(\lambda) = E_{ex,\lambda_{F,det}}(\lambda)\alpha_a(582\text{ nm}) / E_{ex,\lambda_{F,det}=720\text{ nm}}(\lambda = 582\text{ nm})$  of QuasAr1 in pH 8 Tris buffer stored in the dark at room temperature ( $\vartheta = 21\text{--}25\text{ }^\circ\text{C}$ ) are shown in Figure S8 for 50 days of storage and in Figure S9 for 101 days of storage. The curves belong to different fluorescence detection wavelengths  $\lambda_{F,det}$  which are listed in the legends. For comparison the absorption coefficient spectra  $\alpha_a(\lambda)$  of the QuasAr1 sample stored in the dark for 50 days (Figure S8) and for 101 days (Figure S9) are included in the sub-figures.

In the top sub-figures  $E'_{ex}(\lambda)$  curves for  $\lambda_{F,det} = 780\text{ nm}$ ,  $740\text{ nm}$ ,  $700\text{ nm}$ ,  $680\text{ nm}$ , and  $660\text{ nm}$  are presented. The curves belonging to  $\lambda_{F,det} = 780\text{ nm}$ ,  $740\text{ nm}$ , and  $700\text{ nm}$  resemble the absorption coefficient spectrum of Ret\_580 in the wavelength range  $\lambda \geq 310\text{ nm}$ . For  $\lambda < 310\text{ nm}$  the curves are dominated by apoprotein Trp and Tyr absorption and subsequent Förster-type energy transfer to Ret\_580. The curves belonging to  $\lambda_{F,det} = 680\text{ nm}$  and  $660\text{ nm}$  include already emissions from shorter wavelength absorbing retinal isomers.

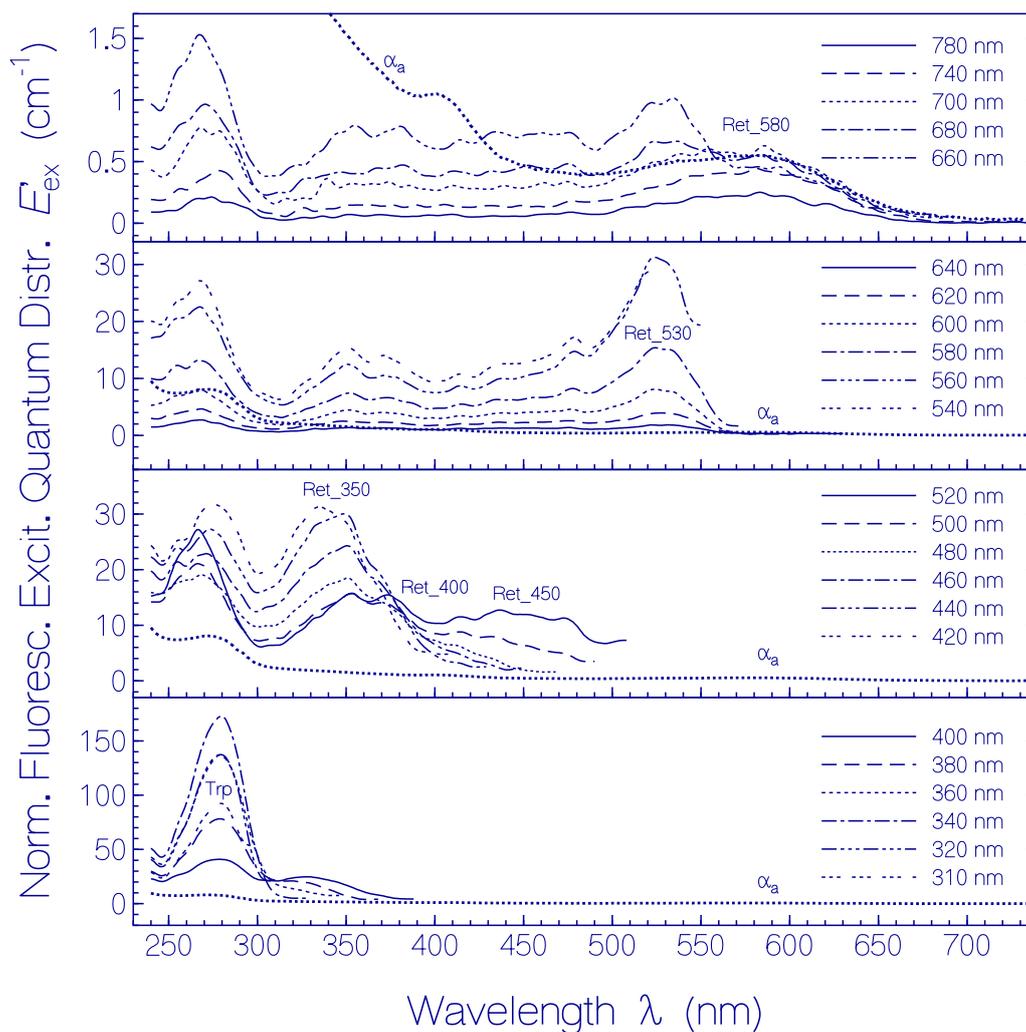


**Figure S8.** Normalized fluorescence excitation quantum distributions  $E'_{ex, \lambda_{F, det}}(\lambda) = E_{ex, \lambda_{F, det}}(\lambda) \times \alpha_a(582 \text{ nm}) / E_{ex, \lambda_{F, det}=720 \text{ nm}}(582 \text{ nm})$  of QuasAr1 in pH 8 Tris buffer stored in the dark at room temperature ( $\vartheta = 21\text{--}25 \text{ }^\circ\text{C}$ ) over a period of 50 days. The fluorescence detection wavelengths  $\lambda_{F, det}$  are listed in the legends.

In the second-top sub-figures  $E'_{ex}(\lambda)$  curves are displayed belonging to  $\lambda_{F, det} = 640 \text{ nm}$ ,  $620 \text{ nm}$ ,  $600 \text{ nm}$ ,  $580 \text{ nm}$ ,  $560 \text{ nm}$  and  $540 \text{ nm}$ . They are dominated by the emission of Ret\_530. For  $\lambda_{F, det} = 580 \text{ nm}$ ,  $\lambda_{F, det} = 560 \text{ nm}$  and  $540 \text{ nm}$  contributions are present from Ret\_450, Ret\_400 and Ret\_350. Below  $\lambda = 300 \text{ nm}$  the curves are dominated by apoprotein Trp and Tyr absorption and subsequent Förster-type energy transfer to Ret\_350, Ret\_400, Ret\_450, and Ret\_530.

In the second-lowest sub-figures normalized fluorescence excitation quantum distributions belonging to  $\lambda_{F, det} = 520 \text{ nm}$  and  $500 \text{ nm}$  are determined by emission from Ret\_450, Ret\_400, Ret\_350 and Förster-type energy transfer from apoprotein Trp and Tyr to Ret\_350, Ret\_400 and Ret\_450. The curves belonging to  $\lambda_{F, det} = 480 \text{ nm}$ ,  $460 \text{ nm}$ ,  $440 \text{ nm}$  and  $420 \text{ nm}$  are determined by emissions from Ret\_400, Ret\_350, and Förster-type energy transfer from Trp and Tyr to Ret\_400 and Ret\_350.

In the bottom sub-figures the normalized fluorescence excitation quantum distributions belonging to  $\lambda_{F, det} = 400 \text{ nm}$  and  $380 \text{ nm}$  indicate fluorescence emission from Ret\_350. The curves belonging to  $\lambda_{F, det} = 360 \text{ nm}$ ,  $340 \text{ nm}$ ,  $320 \text{ nm}$  and  $310 \text{ nm}$  are determined by emission from Trp and Förster-type energy transfer from Tyr to Trp.



**Figure S9.** Normalized fluorescence excitation quantum distributions  $E'_{ex,\lambda_{F,det}}(\lambda) = E_{ex,\lambda_{F,det}}(\lambda) \times \alpha_a(582 \text{ nm}) / E_{ex,\lambda_{F,det}=720 \text{ nm}}(582 \text{ nm})$  of QuasAr1 in pH 8 Tris buffer stored in the dark at room temperature ( $\vartheta = 21\text{--}25 \text{ }^\circ\text{C}$ ) over a period of 101 days. The fluorescence detection wavelengths  $\lambda_{F,det}$  are listed in the legends.

### S9. Primary Photo-isomerization Behavior of Some Rhodopsins

In Table S1 absorption, fluorescence, and first-step photo-isomerization parameters of some microbial rhodopsins (type I rhodopsins) and of an animal rhodopsin (type II rhodopsin) are listed.

**Table S1.** Absorption, fluorescence, and primary photo-isomerization comparison of rhodopsins.

Name	Buffer	Cofactor	$\lambda_{a,max}$ (nm)	$\lambda_{E,max}$ (nm)	$\delta\tilde{\nu}_{Stokes}$ (cm <sup>-1</sup> )	$\phi_F$	$\tau_F$ (ps)	Photo-isomer	Reference
BRh	pH 6.8–7	PRSB <sub>11-cis</sub>	498	640 [40]	4455	$6 \times 10^{-4}$ [86] $1.2 \times 10^{-5}$ [87] $9 \times 10^{-6}$ [40]	0.125–0.33, 1.0–2.4	PRSB <sub>all-trans</sub>	[88]
PR	pH 8	PRSB <sub>all-trans</sub>	521	700	4900	$2.6 \times 10^{-4}$	0.45, 4	PRSB <sub>13-cis</sub>	[41]
BR	pH 7.5	PRSB <sub>all-trans</sub>	568	$755 \pm 10$ [89]	$\approx 4360$	$1 \times 10^{-4}$ [90] $1.3 \times 10^{-4}$ [91]	< 0.15, $\approx 0.45$ 0.2, 0.5 [92] $\approx 0.2, \approx 0.7, \approx 10$ [93]	PRSB <sub>13-cis</sub>	[94]
HR	pH 7	PRSB <sub>all-trans</sub>	578	$\approx 685$	$\approx 2700$	$5 \times 10^{-4}$	5 1.5, 6.6 [95] 1.5, 8.5 [96]	PRSB <sub>13-cis</sub>	[97]
sSRI	pH 6	PRSB <sub>all-trans</sub>	587	$\approx 700$	$\approx 2570$	$1.4 \times 10^{-3}$	5, 33	PRSB <sub>13-cis</sub>	[91]
sSRII	pH 6	PRSB <sub>all-trans</sub>	487				< 0.15, 0.4, 4	PRSB <sub>13-cis</sub>	[91]
pSRII	pH 8	PRSB <sub>all-trans</sub>	497	630	4250	$1.2 \times 10^{-4}$	0.08–0.25, 1.7–2.5 [98]	PRSB <sub>13-cis</sub>	[91]
ChR-2	pH 7.4	PRSB <sub>all-trans</sub>	475	530	2185		0.4	PRSB <sub>13-cis</sub>	[99]
ASR	pH 7.5	PRSB <sub>all-trans</sub>	549				$\approx 0.15$	PRSB <sub>13-cis</sub>	[100]
ASR	pH 7.5	PRSB <sub>13-cis</sub>	537				$\approx 0.72$	PRSB <sub>all-trans</sub>	[100]
HKR1	pH 7.4	RSB <sub>13-cis</sub>	380	$\approx 430$	$\approx 3060$	$1.5 \times 10^{-3}$	5, 60 [101]	RSB <sub>all-trans</sub>	[56]
HKR1	pH 7.4	PRSB <sub>all-trans</sub>	485	$\approx 530$	$\approx 1750$	$2.8 \times 10^{-4}$	0.65, 5 [102]	PRSB <sub>13-cis</sub>	[56]
BeRh	pH 8	PRSB <sub>all-trans</sub>	530	$\approx 640$	$\approx 3250$	$1.7 \times 10^{-4}$	$\approx 1.2$	PRSB <sub>13-cis</sub>	[60]
CaRh	pH 7.3	PRSB <sub>all-trans</sub>	540	$\approx 690$	$\approx 4030$	$1.1 \times 10^{-5}$	0.088	PRSB <sub>13-cis</sub>	[103]
Arch	pH 7.3	PRSB <sub>all-trans</sub> [S20]	558	687	3365	$9 \times 10^{-4}$		PRSB <sub>13-cis</sub> [S20]	[36]
QuasAr1	pH 8	PRSB	580	$\approx 740$	$\approx 3730$	$6.5 \times 10^{-3}$	$\approx 61.5$	PRSB	This work

**Abbreviations:** BRh: bovine rhodopsin (type-II rhodopsin). PR: proteorhodopsin from uncultivated marine  $\gamma$ -proteobacteria. BR: bacteriorhodopsin from *Halobacterium salinarum*. HR: halorhodopsin from *Halobacterium halobium*. sSRI: sensory rhodopsin I from *Halobacterium salinarum*. sSRII: sensory rhodopsin II from *Halobacterium salinarum*.

*Halobacterium salinarum*. pSRII: sensory rhodopsin II from *Natronobacterium pharaonis*. ChR-2: channelrhodopsin 2 from *Chlamydomonas reinhardtii*. ASR: sensory rhodopsin from *Anabaena (Nostoc) sp.* PCC 7120 cyanobacterium. HKR1: histidine kinase rhodopsin 1 from *Chlamydomonas reinhardtii*. BeRh: rhodopsin domain of the rhodopsin-guanylyl cyclase from *Blastocystis emersonii*. CaRh: rhodopsin domain of the rhodopsin-guanylyl cyclase from *Catenaria anguillulae*. Arch: Archaerhodopsin 3 from *Halorubrum sodomense*. QuasAr1: fluorescent voltage sensor 1 of quality superior to Arch from *Halorubrum sodomense*. PRSB: protonated retinal Schiff base. RSB: deprotonated retinal Schiff base.  $\lambda_{a,max}$ : wavelength position of maximum absorption of first absorption band.  $\lambda_{F,max}$ : wavelength position of maximum fluorescence.  $\phi_F$ : fluorescence quantum yield.  $\tau_F$ : fluorescence lifetime.  $\delta\tilde{\nu}_{Stokes} = \lambda_{a,max}^{-1} - \lambda_{F,max}^{-1}$ : Stokes shift = the wavenumber difference between the maximum of the absorption band and the maximum of the corresponding fluorescence band.

