

## Supporting Information

### **Gated Proton Release During Radical Transfer at the Subunit Interface of Ribonucleotide Reductase**

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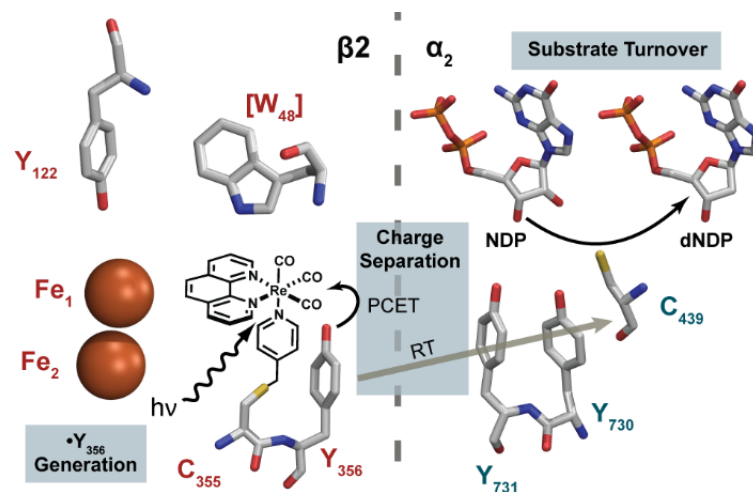
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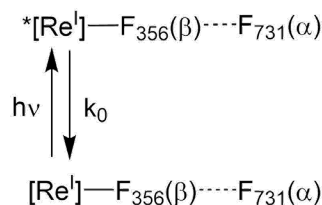
### Photophysical Scheme

Table 1 Entry

Lifetime

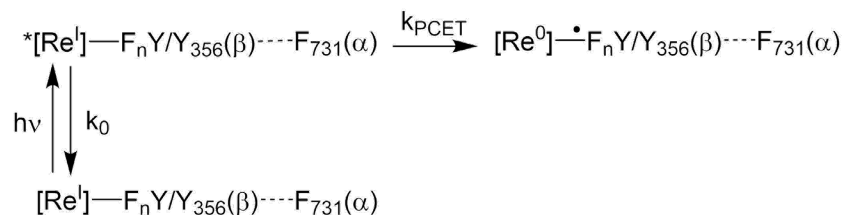
1,5

$$\tau_0 = \left( \frac{1}{k_0} \right)$$



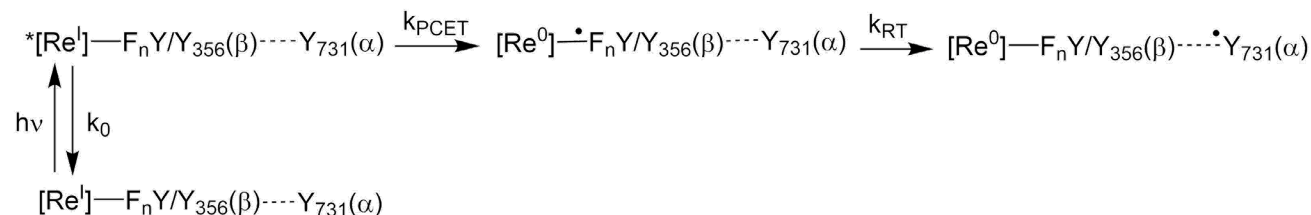
2,3,7,9,11

$$\tau_{\text{obs}} = \left( \frac{1}{k_0 + k_{\text{PCET}}} \right)$$

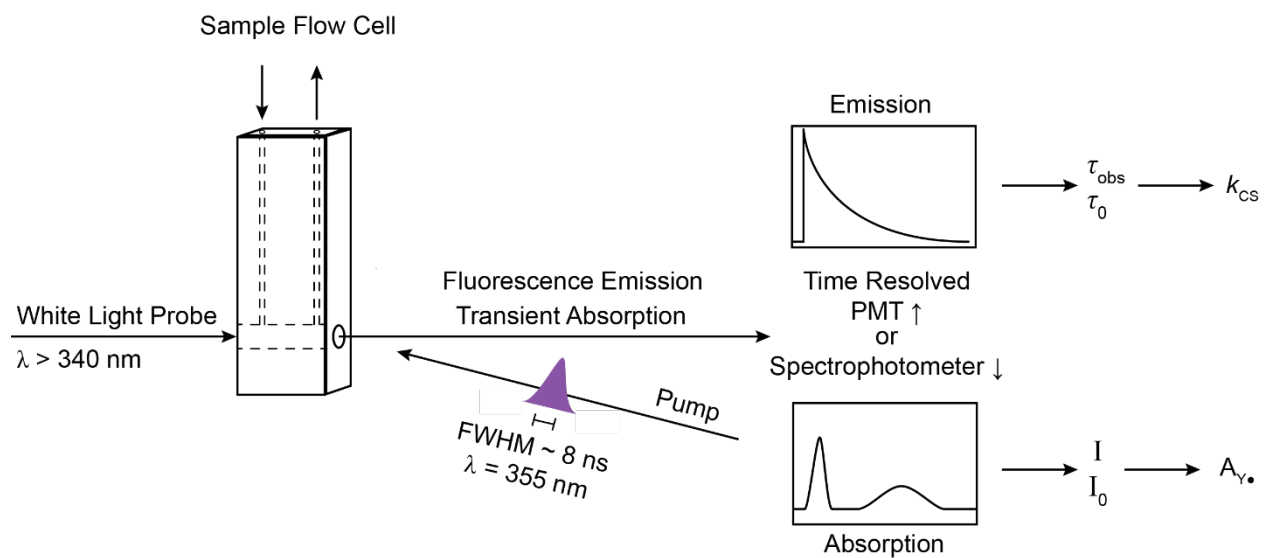


4.8,10,12

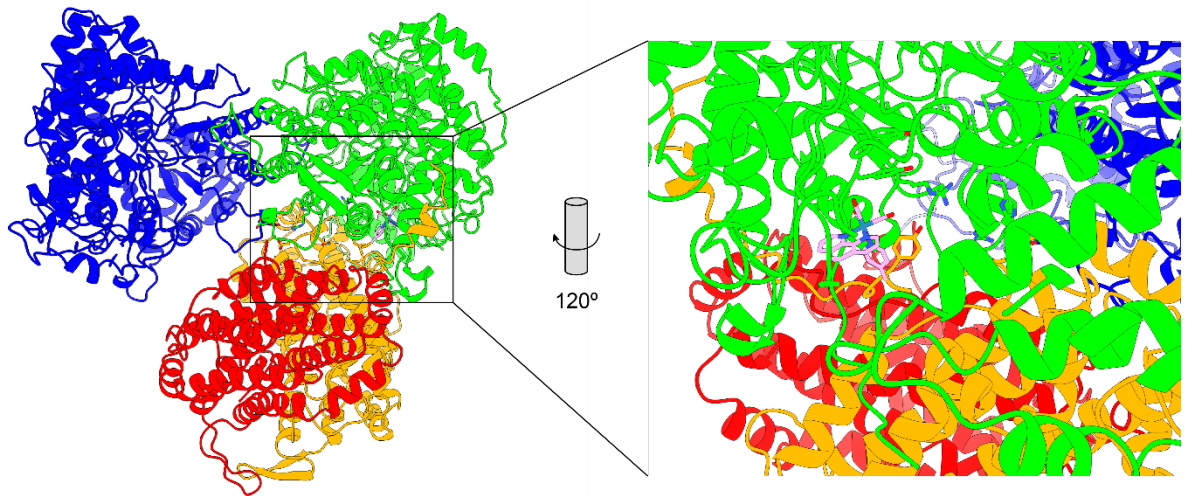
$$\tau_{\text{obs}} = \left( \frac{1}{k_0 + (k_{\text{PCET}} + k_{\text{RT}})} \right)$$



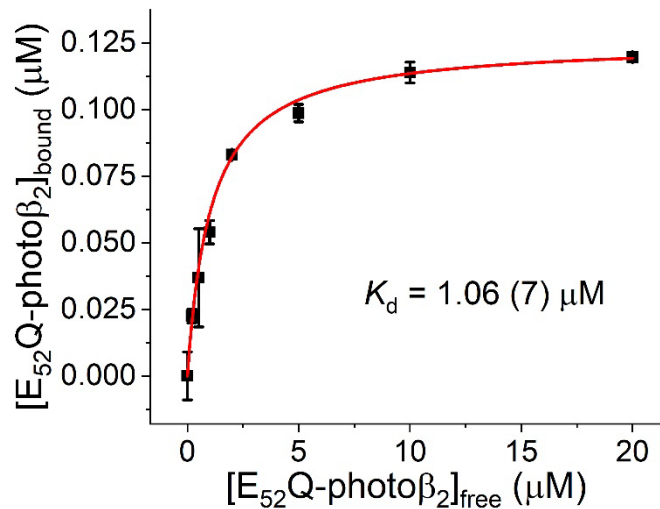
**Figure S1.** (Top) Excited-state reaction pathways after excitation of  $[\text{Re}^I]^*$  in photo $\beta_2$ . Figure 3 is reproduced here for convenience to the reader. (Bottom) description of the lifetimes ( $\tau_0$  and  $\tau_{\text{obs}}$ ) listed in Table 1 of text and accompanying photophysical schemes that describe the lifetimes.



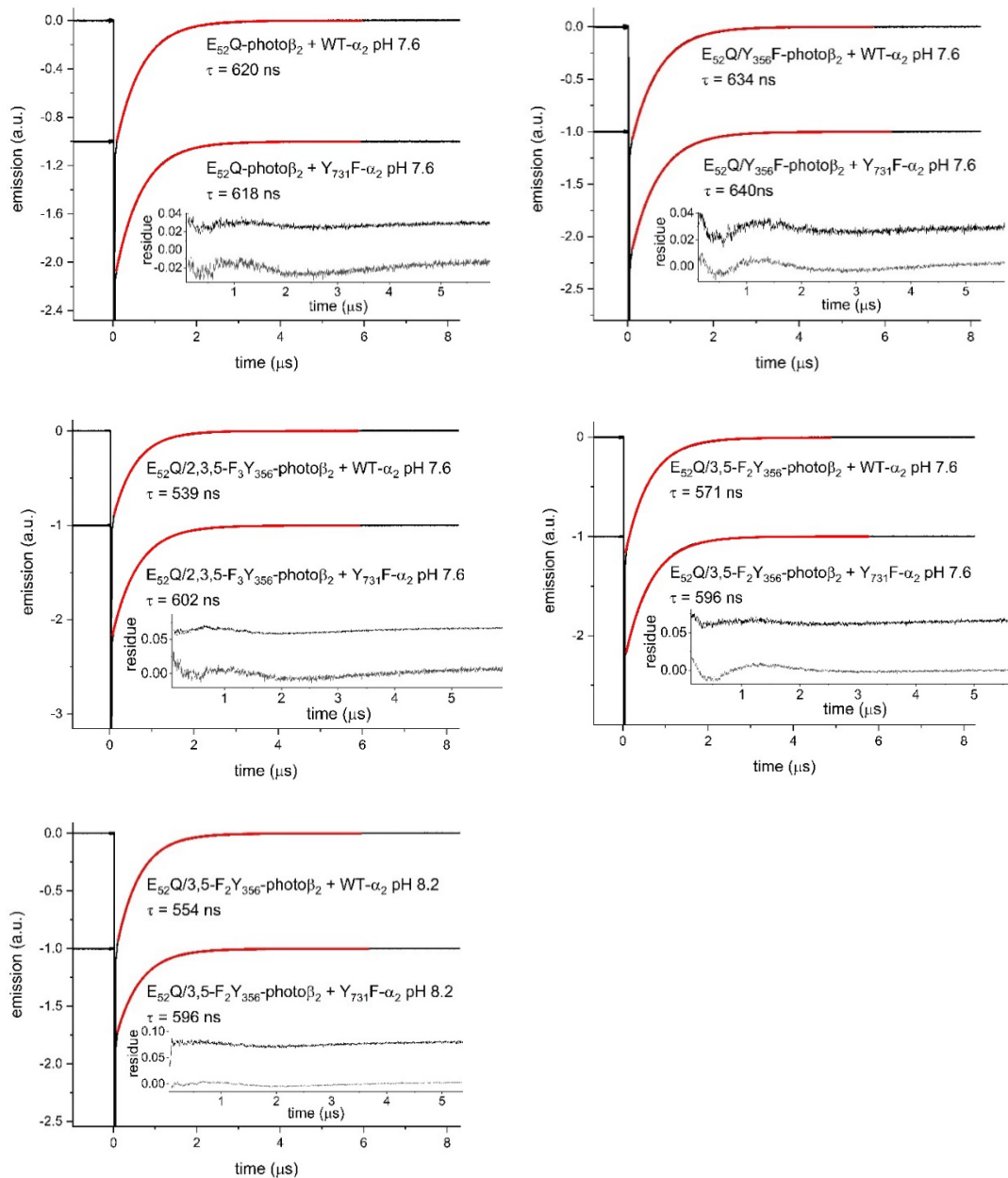
**Figure S2.** Schematic of the emission lifetime and transient absorption experimental setup and data processing.



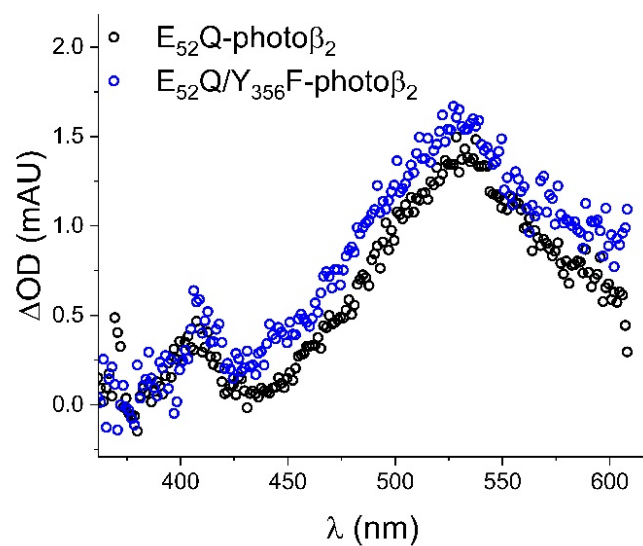
**Figure S3.** Expanded alternative views of the docked [Re] site in the cryo-EM RNR structure.



**Figure S4.**  $K_d$  determination for [Re]-labeled E<sub>52</sub>Q-photoβ<sub>2</sub> with wt α<sub>2</sub>. Conditions as described in subsection “ $K_d$  determination” of the Methods. The  $K_d$  of the wt α<sub>2</sub>β<sub>2</sub> interaction is 0.2 μM,<sup>1</sup> whereas the  $K_d$  of the wt α<sub>2</sub> photoβ<sub>2</sub> interaction is 0.7 μM,<sup>2</sup> the  $K_d$  of the wt α<sub>2</sub> E<sub>52</sub>Q β<sub>2</sub> 0.12 μM<sup>3</sup> and the  $K_d < 0.4$  nM for radical-trapped cryo-EM complex.<sup>4</sup>



**Figure S5.** Representative time traces of the emission  $[Re^I]^*$  and the mono-exponential fitting with the residual difference shown in the figure inset. The emission kinetics were measured with  $5 \mu\text{M } \alpha_2$ ,  $2 \mu\text{M } E_{52}Q\text{-photo}\beta_2$  variants,  $1 \text{ mM CDP}$ ,  $3 \text{ mM ATP}$  in assay buffer and lifetimes reported represent the average of three independent measurements consisting of 100 traces each.



**Figure S6.** Transient absorption spectra of  $E_{52}\text{Q-photo}\beta_2$  and  $E_{52}\text{Q/Y}_{356}\text{F-photo}\beta_2$ . Conditions as described in Transient absorption spectroscopy subsection of the Methods.



## References

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- (4) Kang, G.; Taguchi, A. T.; Stubbe, J.; Drennan, C. L. Structure of a Trapped Radical Transfer Pathway within a Ribonucleotide Reductase Holocomplex. *Science* **2020**, *368*, 424–427.