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Supporting Material

Simultaneous measurements of solvent dynamics and functional kinetics in a light-activated enzyme.

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Table S1.

Thermodynamic parameters extracted from TDAM data of Fig.3 and 4a.

	S_{25}-1	S_{25}-2	S_{40}-1	(S_{25}-1 + S_{25}-4)[&]
ν_1 [s^{-1}][*]	24.1	65.3	155.2	63
ΔH_1 [kJ.mol^{-1}][%]	14.15	15.51	16.59	15.38
ν_2 [s^{-1}]	3.19e+16	6.85e+16	1.15e+12[$]	-
ΔH_2 [kJ.mol^{-1}]	73.7	76.2	55.5[$]	-
ν_{VTF} [s^{-1}]	1.54e+09	2.50e+09	1.07e+08[$]	-
E_{VTF} [kJ.mol^{-1}]	27.7	27.1	28.6[$]	-
T_{VTF} [K]	75.9	82.7	54.0[$]	-

[*] For a photo-activated process, ν_1 is proportional to the intensity of the actinic light.

[%] The value of ΔH_1 reported in the text corresponds to the average between the three samples S_{25}-1, S_{25}-2 and S_{40}-1.

[&] Parameters obtained from global fitting to both data sets S_{25}-1 (G = 100 K/hour) and S_{25}-4 (G = 150 K/hour).

[$] Parameters considered less reliable due to the extended rate distribution in S_{40}-1 possibly caused by solvent de-mixing.

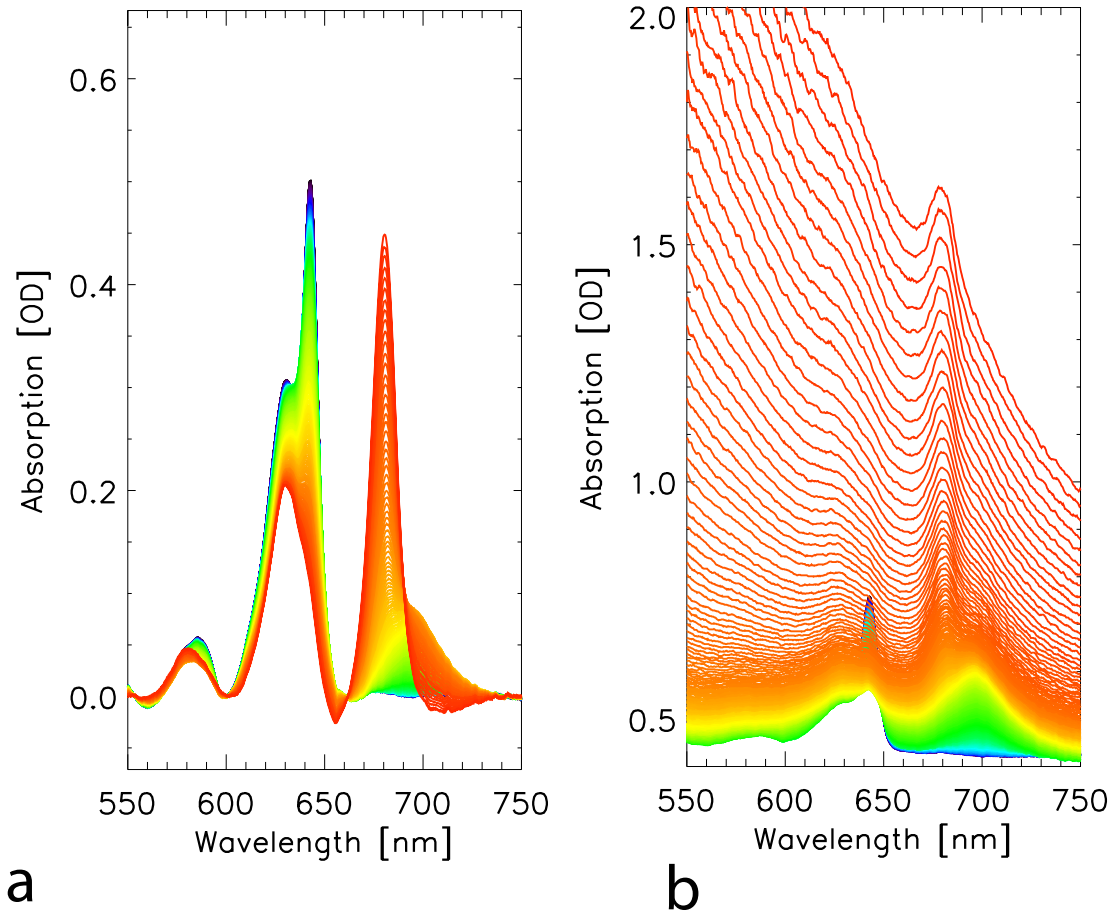

Fig. S1: Spectroscopic “movie” of the POR reaction pathway in TDAM mode. (a) Absorption spectra evolve from blue to red as the temperature increases at a rate of 100 K/hour. (b) Raw TDAM data showing the temperature-dependant baseline increase associated with light scattering from developing ice clusters.

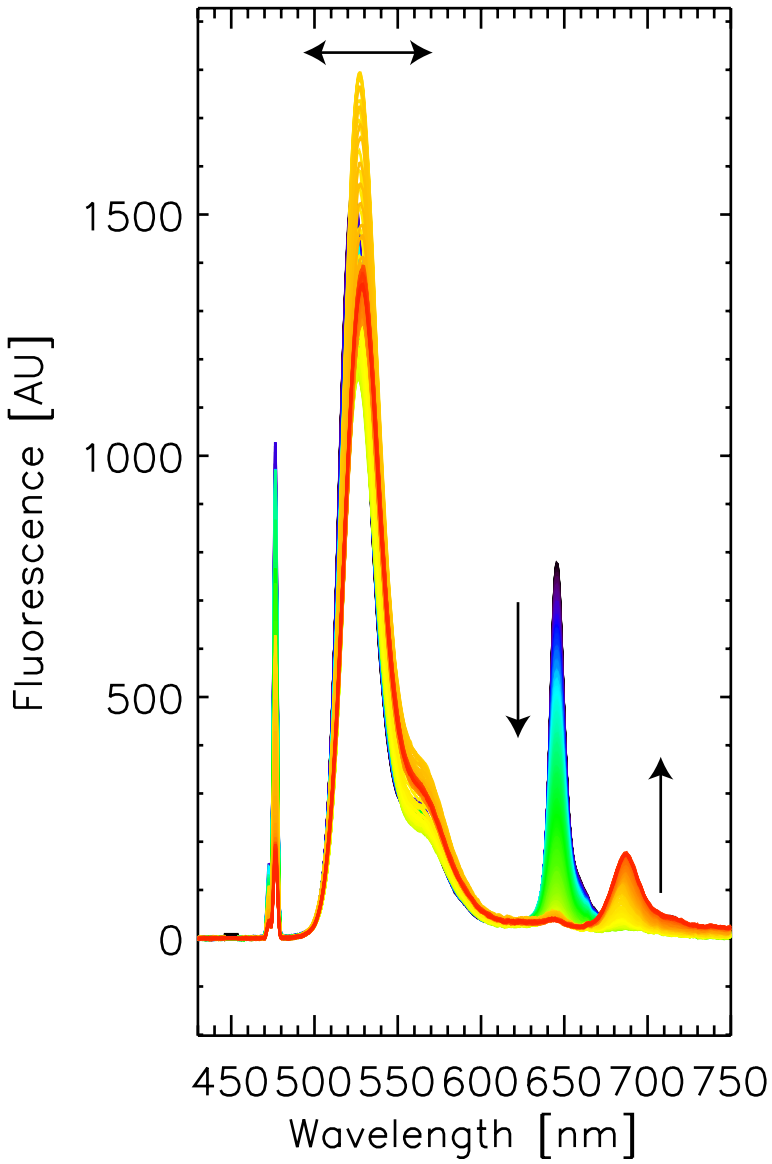

Fig. S2: Spectroscopic “movie” of the POR reaction pathway in TDFM mode. The evolution of the fluorescence emission of Oregon Green (horizontal arrow) can be seen in parallel with the POR catalytic cycle (vertical arrows).

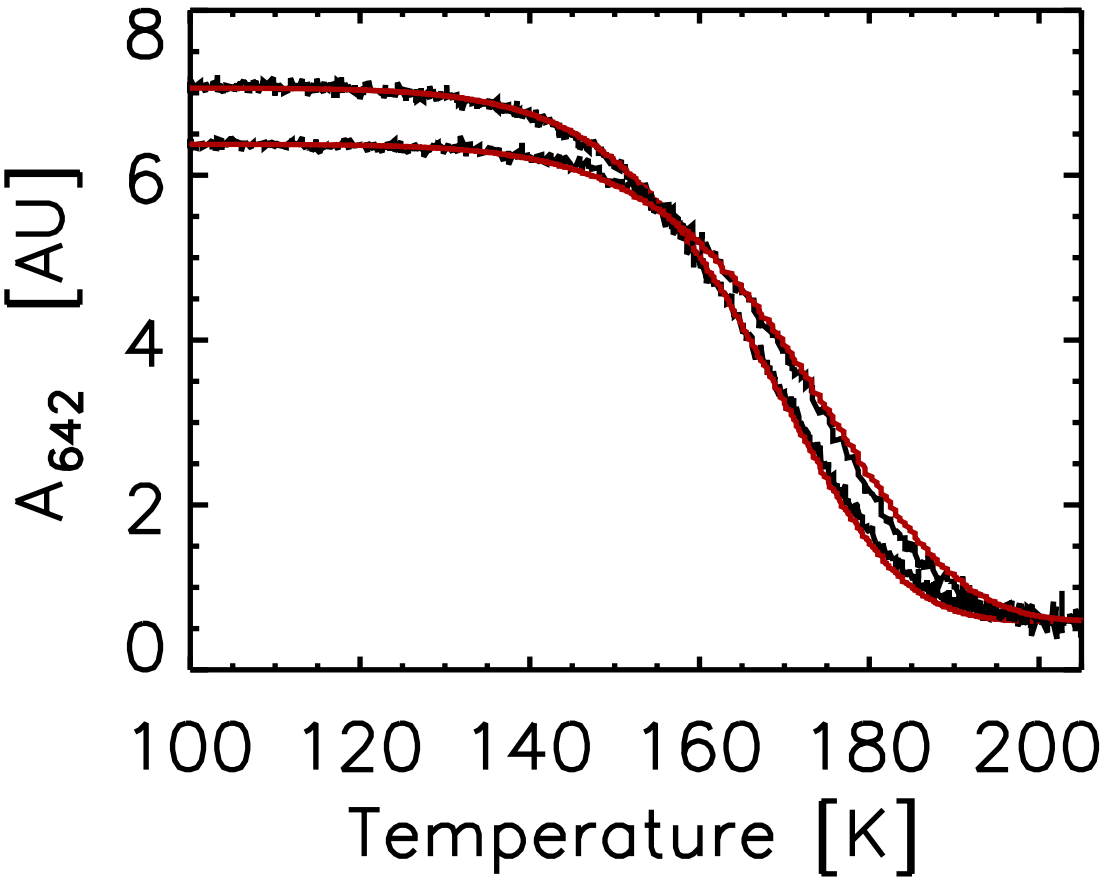

Fig. S3: Global fit of the A642 decay from two data sets recorded with different temperature ramps (100 K/hour, and 150 K/hour).

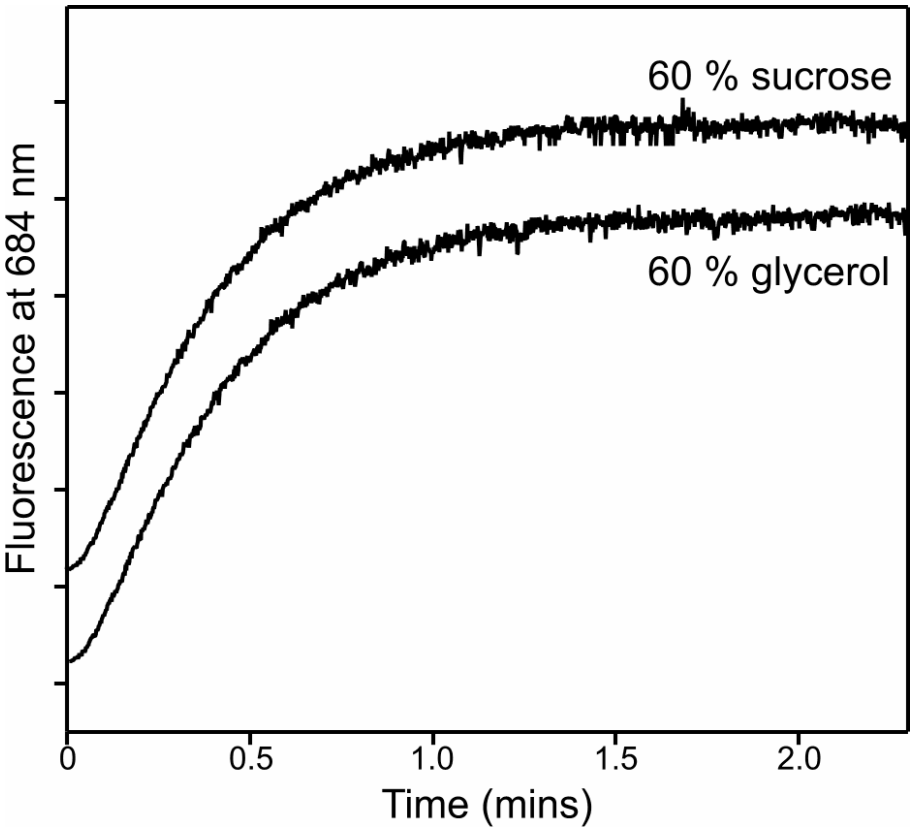

Fig. S4: Kinetics of the 2nd step of the POR reaction measured in 60 % glycerol and 60 % sucrose solutions. The rate of increase in fluorescence at 684 nm was measured at 230 K as previously described (ref 26) for samples containing 1.2 μM PChlide, 30 μM POR and 200 μM NADPH, using either 60 % glycerol or 60 % sucrose as a cryogenic buffer. The traces are offset for ease of clarification. Similar rates are observed for both cryogenic buffers (0.035 ± 0.003 s^{-1} for 60 % glycerol and 0.039 ± 0.004 s^{-1} for 60 % sucrose), indicating that this step is not influenced by the nature of the viscogen.