

pH and Potential Transients of the bc_1 Complex Co-reconstituted in Proteo- Lipobeads with the Reaction Center from *Rb.* *sphaeroides*

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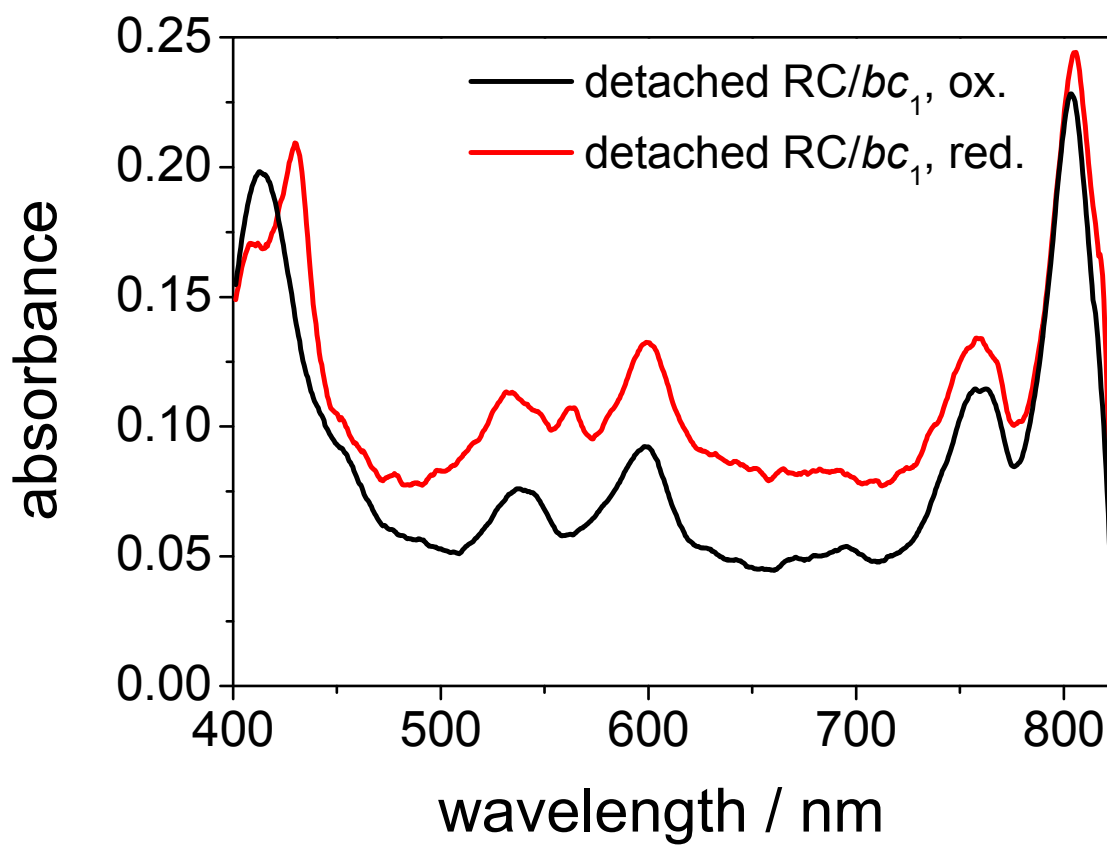


Figure S1. UV/Vis absorption spectra of the 1:1 mixture of RC with bc_1 complexes before and after reduction with sodium hydrosulfite, detached from NTA-modified beads by imidazole.

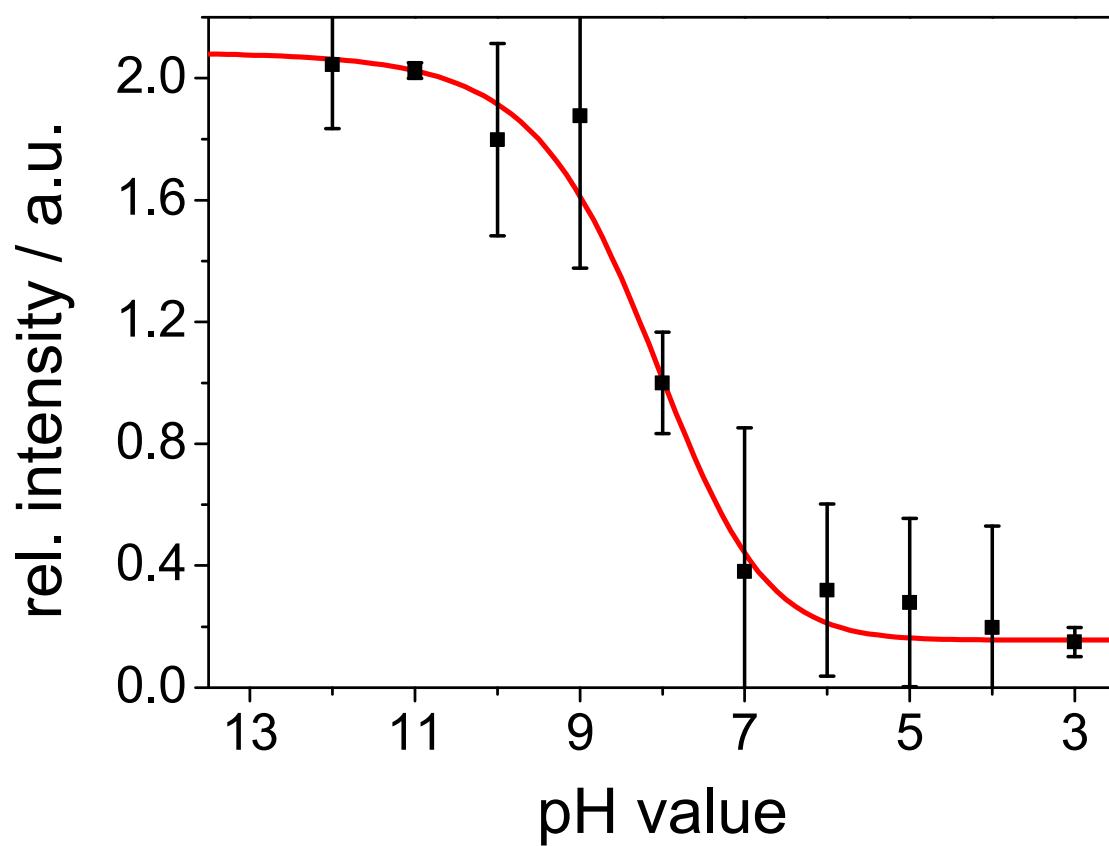


Figure S2. Calibration plot of PLBs labeled with fluorescein-DHPE as a function of pH. Fluorescence emission intensities were measured of PLBs labeled with fluorescein-DHPE immersed in KCl solution (35 mM, 5 mM citrate, titrated to pH values 3-10).

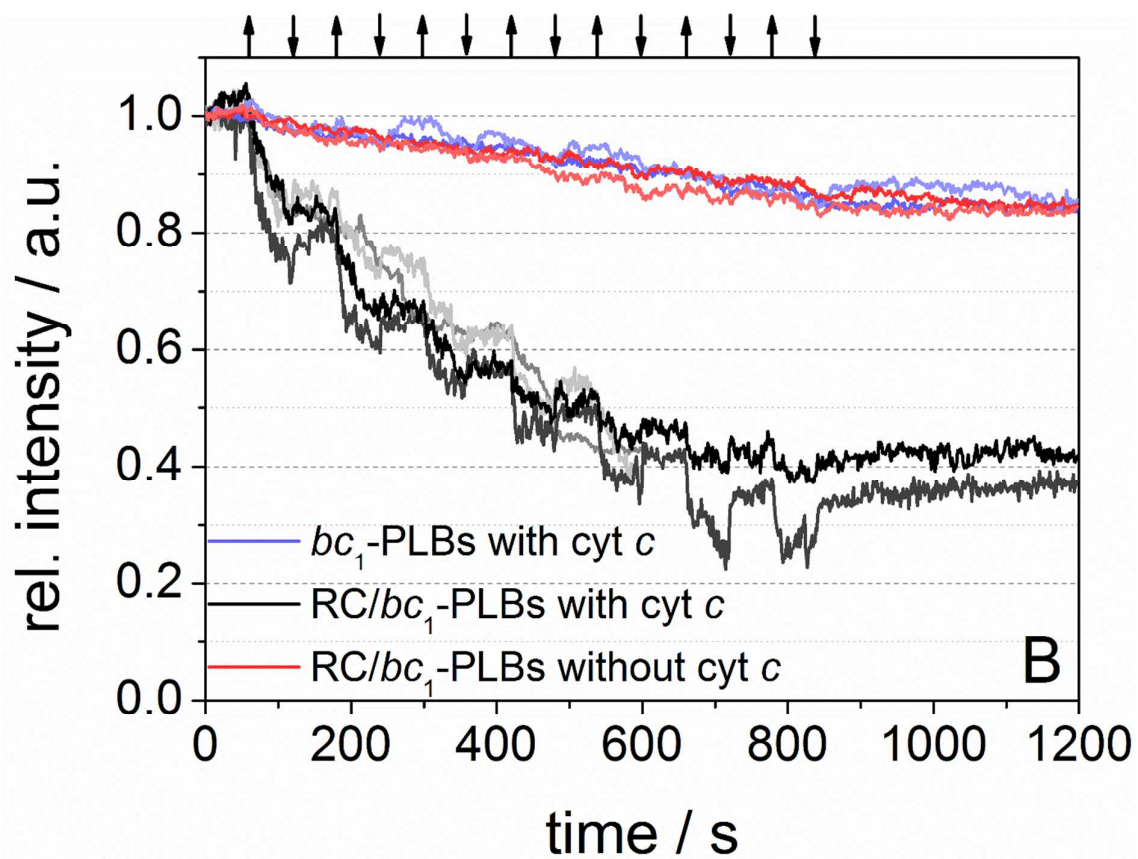
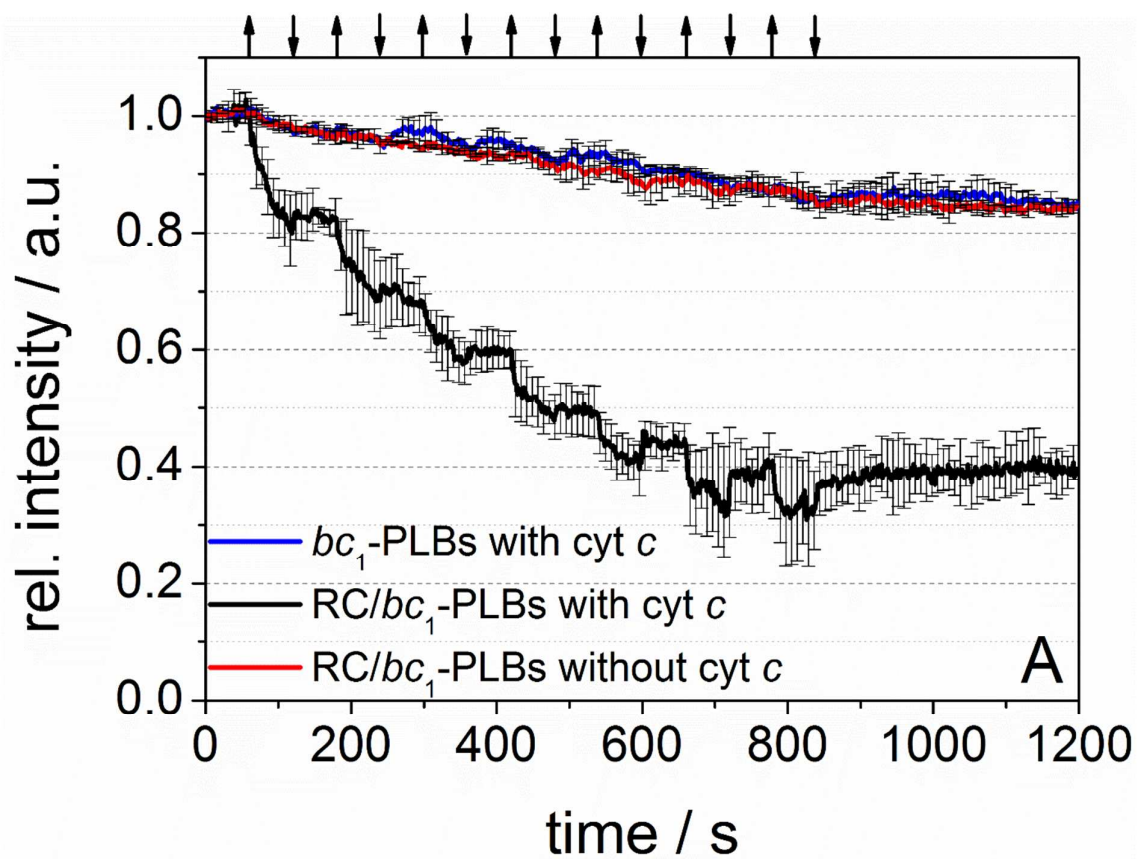


Figure S3. Reproducibility of the data. (A) Selected curves of Fig. 3 with mean value and standard deviation. (B) Single measurements. (RC and bc_1 complexes co-reconstituted with Q-10 within PLBs labeled with fluorescein-DHPE, cyt c added to the aqueous solution (black) and control experiments without cyt c (red) and with bc_1 alone (blue). Arrows indicate when the halogen lamp is switched on (\uparrow) and off (\downarrow .)

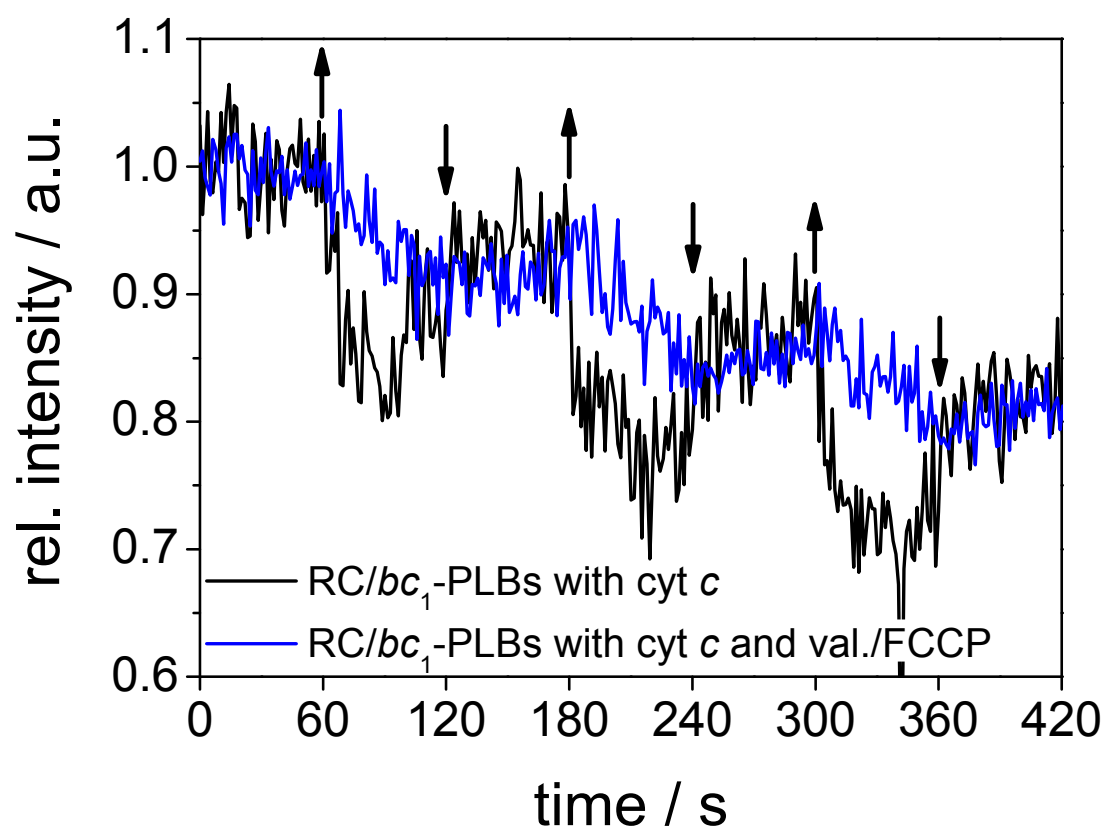


Figure S4. RC and bc_1 complexes co-reconstituted in PLBs with Q-10 – cytochrome c was added to the aqueous solution – illuminated with the halogen lamp in the absence (black) and presence valinomycin and FCCP (blue), respectively, labeled with di-4-ANBDQBS. Arrows indicate when the halogen lamp is switched on (\uparrow) and off (\downarrow).

Evaluation of pH-transients

pH vs. time plots (Fig. 3B) after corrections for bleaching effects (subtraction of the control experiment without RC) as well as for signal offsets due to switching were fitted to eq (1).

$$pH(t) = y_0 + Ae^{R_0 t} \quad (1)$$

From the first derivative of eq (1)

$$pH'(t) = R_0 A e^{R_0 t} \quad (2)$$

the initial rate $\frac{\Delta pH}{dt}$ was obtained.

Table S1. Parameters obtained from the fitting

	R₀	A	y₀	dpH/dt_{ini}
- valinomycin	-0.023	1.181	7.663	0.006
+ valinomycin	-0.024	3.153	7.120	0.017

Number of bc₁ complexes bound to 12.3 μL PLB pellet

With 4.6×10^{-10} mol *bc₁* complexes bound to 250 μL PLB pellet out of which 12.3 μL were used for the measurement, the number of *bc₁* calculates to

$$4.6 \times 10^{-10} \times 6.022 \times 10^{23} = 2.76 \times 10^{14}$$

$$\frac{12.3}{250} \times 2.76 \times 10^{14} = 1.36 \times 10^{13}$$

Calculation of the number of protons per bc₁ complex bound to Tris buffer at the initial proton release rate

With $pK_a = 8.2$ of Tris buffer and a $dpH/dt_{ini} = 0.0063 \text{ s}^{-1}$ the percentage of protonated Tris molecules was obtained using the Henderson-Hasselbalch equation to be 0.33 %.

$$pH = pK_a + \log_{10} \frac{[A^-]}{[HA]} \quad (3)$$

$$10^{pK_a - pH} = \frac{[HA]}{[A^-]} \quad (4)$$

From this the number of protons bound to 388 μL Tris-buffer (5 mmol/L) calculates to:

$$0.0033 \times 5 \times 10^{-3} \times 387.7 \times 10^{-6} \times 6,022 \times 10^{23} = 3.89 \times 10^{15}$$

$$\frac{3.89 \times 10^{15}}{1.36 \times 10^{13}} = 286$$

The number of protons bound to fluorescein DHPE ($pK_a = 6.7^1$) are in the order of magnitude of 10^{12} , negligible in comparison.

REFERENCES

(1) Martin, M. M.; Lindqvist, L. The pH dependence of fluorescein fluorescence. *Journal of Luminescence* **1975**, *10*, 381-390.