Photo-induced dynamics of the heme centers in the cytochrome bc_1

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Supplementary Information

I. Femtosecond transient signal of the bc_1 complex after partial reduction by decylubiquinole, before addition of dithionite.

Before the addition of dithionite, the c-hemes of the bc_1 complexes have been reduced by adding solution of decylubiquinole to a final concentration of 0.8 mM. At low bc1 concentrations, decylubiquinole is able to fully reduce the c_1-hemes as well as the hemes b_L. Within our experimental conditions and as illustrated in Figure 2 (main article), decylubiquinole effectively reduced the c_1-hemes while the *b*-hemes were left oxidized.

In the following conditions, transient data were recorded as explained in the main article, resulting in the following time-wavelength surface (Figure S1).

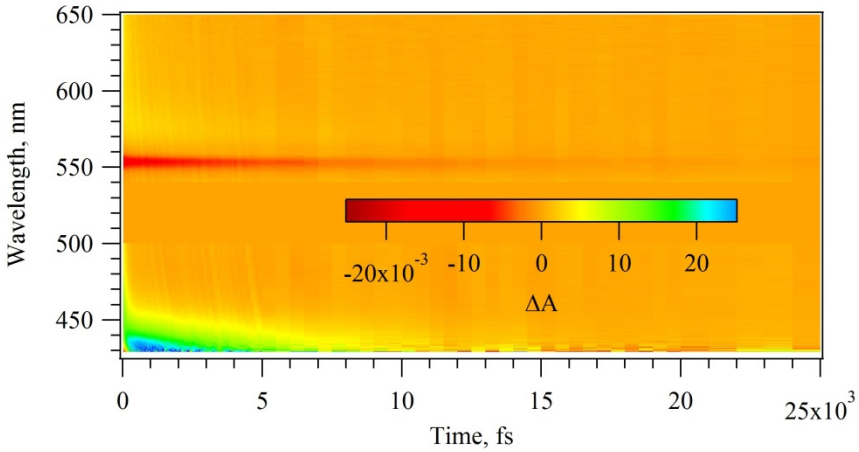

Figure S1: Transient data taken before addition of dithionite, while c_1-hemes have been reduced by decylubiquinole. The scattering region around the 523-nm excitation is set to zero.

Indeed, only the 553-nm bleach, representative of the ferrous c_1-heme alpha band, is present (red trace in Figure S1), while no specific signals from the ferrous *b*-hemes are discernable at 562 nm. The set of selected kinetics was globally fitted with a minimum of 3 decay components (not shown). The latters are crosschecked with an SVD analysis of the surface (Figure S1) and used to generate the following DAS (Figure S2).

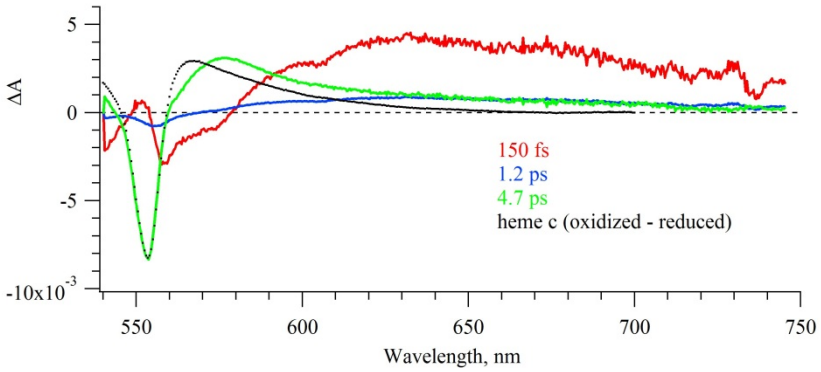

Figure S2: DAS of the c_1-hemes α-band spectral region before addition of dithionite. The oxidized minus reduced spectrum of the c_1-heme, normalized at the maximum bleach amplitude, is superimposed as reference (black dotted line).

The DAS present in Figure S2 corresponds well, in terms of spectral features and associated decay components, to the one resulting from the oxidized bc_1 complex, i.e. after reduction of the *b*-hemes by dithionite and after oxidation of the same *b*-hemes by oxygen Figure 6A. The small differences between the two set of data are within the fitting allowances; such that the exponential decay values of one set could be used to fit the other without alteration of the main DAS features. This correspondence is supporting that the c_1-hemes are left unaffected by both the addition of dithionite and by the presence of molecular oxygen.

II. Femtosecond transient signal of the bc_1 complex after reduction by dithionite.

In order to directly evaluate the respective involvement of each heme types in their photo-dissociation and photo-oxidation, we proceed to the analysis of the data while both heme types are reduced. The goal is to base our calculations on a data set that underwent the least manipulations. Global and SVD analysis was consequently done on the time-wavelength surface shown in Figure 3A, while both the b and c-hemes are reduced via dithionite. The generated DAS is shown below (Figure S3). Because of the correspondence between the exponential decay components present in the *b*-hemes (190 fs, 1.6 ps and 5.4 ps) and in the c_1-hemes (300 fs and 6.8 ps), the data was fitted with a minimum of three components, rather than five. The aim of this analysis was solely to extract the DAS corresponding to the longest mixed *b*- and c_1-hemes ps-response. Indeed, the generated 6.9-ps DAS is cleaner than typical extracted spectra at a particular delay time, devoid of any short interfering processes and consequently better suitable for fitting. The validity of the DAS is illustrated by its correspondence with the superimposed spectra taken at 1 ps after excitation (Figure S3). The 6.9-ps DAS is fitted in terms of the heme *b* and c_1 contributions in the following Figure S5B.

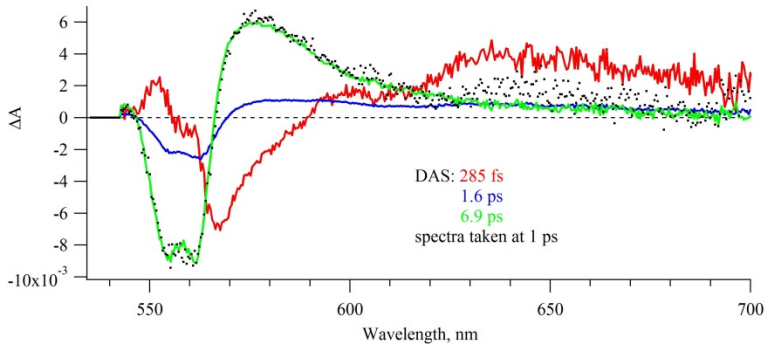

Figure S3 : DAS in the α-band spectral region after addition of dithionite, while both the c_1- an *b*-hemes are reduced. The spectrum taken at 1 ps after laser excitation is superimposed as reference (black dotts).

III. Fitting procedure of the different spectra

In order to evaluate the contribution of the *b*- and c_1-heme to the different steady state absorption and transient signals, we fit the different spectra using the (reduced – oxidized) spectra of both heme-types. To acquire these

reference (reduced – oxidized) spectra, we proceed to the successive reduction by ascorbate and dithionite of the previously oxidized bc_1 complexes, as shown in Figure S4. In this experiment, the concentration of the bc_1 complexes is kept low to ensure for efficient reduction abilities of both ascorbate and dithionite.

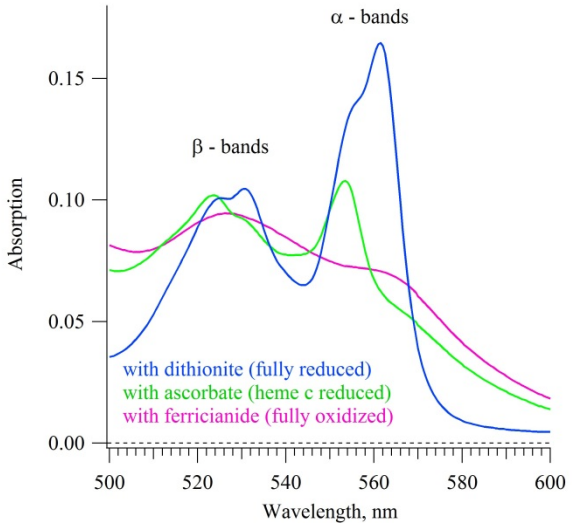

Figure S4: Static absorption spectrum of the sequential reduction the Cyt bc_1 dimer complex: fully reduced with ferricianide (pink), after partial reduction by ascorbate (green) and after full reduction by dithionite (blue).

The (reduced – oxidized) spectra of the *b*- and c_1-heme are obtained by subtracting the ascrobate from the dithionite spectra and the ferricianide from the ascorbate spectra, respectively. Assuming that ascorbate and dithionite fully reduce the c_1- and *b*-hemes, respectively, the amplitude of each is taken as being equal to unity in the following fits. Similarly, the ferricianide spectrum is assumed to represent the fully oxidized complex and its amplitude is also taken as equal to unity in the following fits. The coefficients in Table S1 therefore correspond to the amplitude of the different spectra relative to their amplitude in Figure S4.

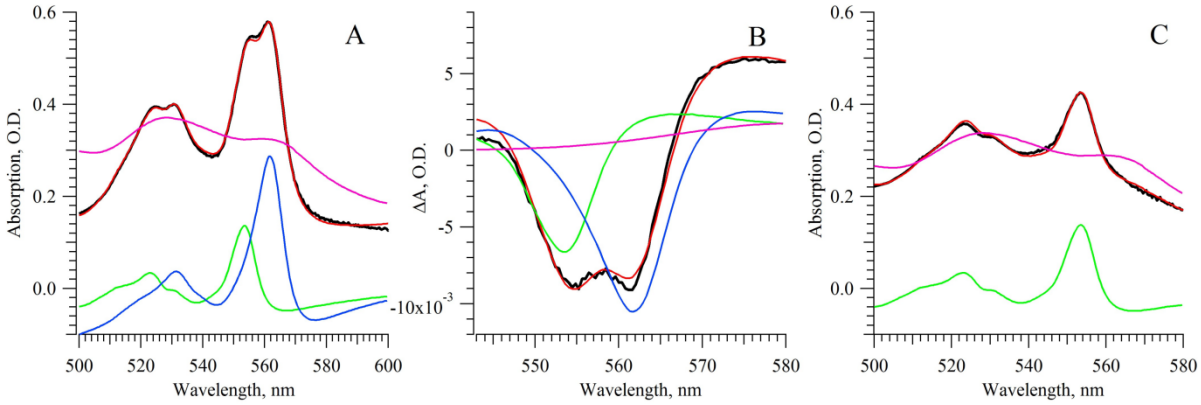

Figure S5: (Same as Figure 8) The black curves represent (A) the static absorption spectrum after addition of dithionite, (B) the DAS corresponding to the longest ps component resulting from the combined analysis of the *b*- and c_1-heme transient signals as described in text; while both are reduced and (C) the static absorption spectrum after full oxidation of the *b*-hemes via oxygen. Superimposed are the fits (red) and their components: the (reduced – oxidized) heme c_1 (green) and heme *b* (blue) spectrum. The additional background (pink) used in each fit is described subsequently.

Since the absorption spectra (Figure S5A and C) are obtained via the probe beam through the capillary, each contains a small additional background due to the scattering of the light. This additional background is assumed to be broad and featureless and is accounted for as a second order polynomial whose coefficients are indicated in Table S1. In each absorption plot of Figure S5, the pink curve represents the sum of the oxidized bc_1 spectrum and the

additional polynomial. The DAS represented in Figure S5B is however expected to contain some ESA signals. Assumed to be broad, featureless and positive it is accounted for by a Gaussian as described in Table S1.

Table S1 : Amplitude of the heme c_1 and *b* components resulting from the fitting of each plot in Figure 5.

	Figure S5A	Figure S5B	Figure S5C
heme c_1*	$A_{heme\ c} = 3.88$	$0.189 = A_{heme\ c}$ x 4.9%	3.93
heme *b**	$A_{heme\ b} = 2.8$	$0.103 = A_{heme\ b}$ x 3.7%	-
Oxidized bc_1	3.41	-	3.45
function	2nd order polynomial: $y = 0.001 \cdot x - 0.49$	Gaussian: A=0.0018, pos=581 nm, FWHM=32 nm	2nd order polynomial: $y = 0.0096 \cdot x - 0.49$

* (reduced-oxidized) spectrum amplitude

Assuming that the c_1-hemes are fully reduced (100%) during the experiment, the relative amplitude of both heme types in the fit of Figure S5A indicates that only ~70% of the *b*-hemes are reduced. Note that the ~1% difference between the c_1-hemes amplitude coefficient of the fits of Figure S5A and Figure S5C in Table S1 is within the errors allowances of the fits and illustrates that the c_1-hemes were left intact during the course of the experiment. Similarly, the amplitude coefficient of the oxidized bc_1 spectra in both Figure S5A and C implies that no additional background appeared due to denaturation of the complexes and points to the fact that the complexes in their entirety were left intact during the course of the experiment.