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Origin of the F685 and F695 fluorescence in Photosystem II

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Abstract

The emission spectra of CP47-RC and core complexes of Photosystem II (PS II) were measured at different temperatures and excitation wavelengths in order to establish the origin of the emission and the role of the core antenna in the energy transfer and charge separation processes in PS II. Both types of particles reveal strong dependences of spectral shape and yield on temperature. The results indicate that the well-known F-695 emission at 77 K arises from excitations that are trapped on a red-absorbing CP47 chlorophyll, whereas the F-685 nm emission at 77 K arises from excitations that are transferred slowly from 683 nm states in CP47 and CP43 to the RC, where they are trapped by charge separation. We conclude that F-695 at 77 K originates from the low-energy part of the inhomogeneous distribution of the 690 nm absorbing chlorophyll of CP47, while at 4 K the fluorescence originates from the complete distribution of the 690 nm chlorophyll of CP47 and from the low-energy part of the inhomogeneous distribution of one or more CP43 chlorophylls.

Introduction

Photosystem II (PS II) is a multisubunit pigment-protein complex embedded in the thylakoid membrane of green plants, algae and cyanobacteria. Photochemical charge separation and secondary electron transport take place in the PS II reaction center (RC), which is surrounded by the core antenna proteins CP47 and CP43 (Dekker and Boekema 2004). The primary function of these core antenna proteins is to harvest light and to efficiently deliver the energy of the absorbed light to the RC. Recently published X-ray structures reported for cyanobacterial PS II cores (Zouni et al. 2001; Kamiya and Shen 2003; Ferreira et al. 2004) revealed the presence of 8, 14 and 16 chlorophyll and pheophytin molecules within the RC, CP43 and CP47 subunits, respectively, participating in energy transfer and/or charge separation.

At physiological temperatures, charge separation in PS II is usually explained in terms of the excited state radical pair equilibrium model (van Grondelle 1985; Schatz et al. 1988). This model successfully explains the relatively long fluorescence lifetimes observed in purified RC and CP47-RC particles (Andrizhiyevskaya et al. 2004; van Mourik et al. 2004). In both studies three reversible radical pair states had to be assumed, of which the first one had about the same energy as the equilibrated excitation energy of the RC. Furthermore, experimental evidence was obtained that suggested that the kinetics of charge separa-

tion was not limited by the energy transfer between CP47 and the RC, but by the formation of the secondary and tertiary radical pairs (Andrizhiyevskaya et al. 2004). At low temperatures, however, much less is known about the dynamics of PS II. The low-temperature emission spectrum of the PS II core complex is thought to originate from long-wavelength chlorophylls in the core antenna: 77 K emission at 695 nm arises from one 690 nm chlorophyll in CP47, whereas 77 K emission at 685 nm arises from several ~683 nm chlorophylls in CP43 and/or CP47 (van Dorssen et al. 1987a, b).

In this work, we analyze the temperature and excitation wavelength dependence of fluorescence spectra of CP47-RC and core complexes to further elucidate the role of the core antenna in the processes of energy transfer and charge separation in PS II, and conclude that the characteristic F-695 and F-685 emissions of PS II have different origins.

Materials and methods

PSII RCs were purified from spinach Tris-washed 'BBY' grana membranes as described elsewhere (van Leeuwen et al. 1991). CP47-RC complexes were purified from spinach as described elsewhere (Dekker et al. 1989). PS II core complexes from *Synechocystis* 6803 were prepared according to the methods described earlier (Rögner et al. 1990; Tang and Diner 1994).

For the spectroscopic measurements, CP47-RC samples were diluted in a buffer containing 20 mM Bis-Tris (pH 6.5), 10 mM $MgCl_2$, 10 mM $CaCl_2$ and 0.03% *n*-dodecyl-β-D-maltoside (β-DM), which was supplemented with 66% (w/w) glycerol. PS II cores were diluted in a buffer containing 50 mM MES (pH 6.1), 5 mM $MgCl_2$, 20 mM $CaCl_2$ and 0.03% *n*-dodecyl-β-D-maltoside (β-DM) and 66% (w/w) glycerol.

The optical density of the samples used for the low temperature (LT) absorption and fluorescence measurements was about 0.7 and 0.1 cm^{-1}, respectively, at the Q_y absorption maximum. The absorption of the samples was compared before and after measurement. No sample degradation was observed.

The absorption spectrum of the 5 K was recorded with a spectral resolution of 0.5 nm on a home-built spectrophotometer as described in (Pålsson et al. 1998).

Fluorescence spectra were measured with a cooled CCD camera (Cromex ChomCam), equipped with a 1/2-m spectrograph (Chromex 500IS). For non-selective fluorescence measurements, a tungsten halogen lamp in combination with an interference filter (with a transmission maximum at 420 nm and a fwhm of 20 nm) was used as excitation source. For site-selective fluorescence measurements, a cw dye laser (Coherent CR599) with DCM dye, pumped by an argon ion laser (Coherent Innova 310), was used. The excitation power was 300 $\mu W/cm^2$, the illumination time was 10 s and the spectral resolution was 0.5 nm. The emission was detected at magic angle (54.7°) with respect to the polarization of the excitation light.

Results

The 4 K emission spectra of RC, CP47-RC and PS II core complexes obtained upon non-selective 420 nm excitation are shown in Figure 1a. The emission of the RC complex peaks at 683.7 nm and is blue-shifted relative to the other two complexes. The CP47-RC emission peaks at 691 nm and has a fwhm = 12.5 nm, similar to the isolated CP47 complex (Groot et al. 1995). The emission of the PS II core peaks at 687.5 nm and has a shoulder at 691 nm, fwhm = 14 nm. From a comparison of the shapes of spectra in Figure 1a it is clear that at 4 K the contribution of the RC emission to the total emission of the CP47-RC and the PS II core complexes is negligibly small. Otherwise the spectra of CP47-RC and core would have a pronounced shoulder at around 684 nm. We note that the 687.5 nm peak in the PS II core emission spectrum can in principle arise from the RC if the red-most RC chlorophyll in the PS II core complex is red-shifted compared to that in the RC complex. It is however unlikely that such a red-shift occurs (Dekker and Van Grondelle 2000), though the distribution between 680 and 684 nm states can be shifted to 683 nm states in more intact complexes (Hillmann et al. 1995).

Increasing the temperature from 4 to 77 K results in a shift of the emission maximum of CP47-RC (Figure 1b) and the core complex

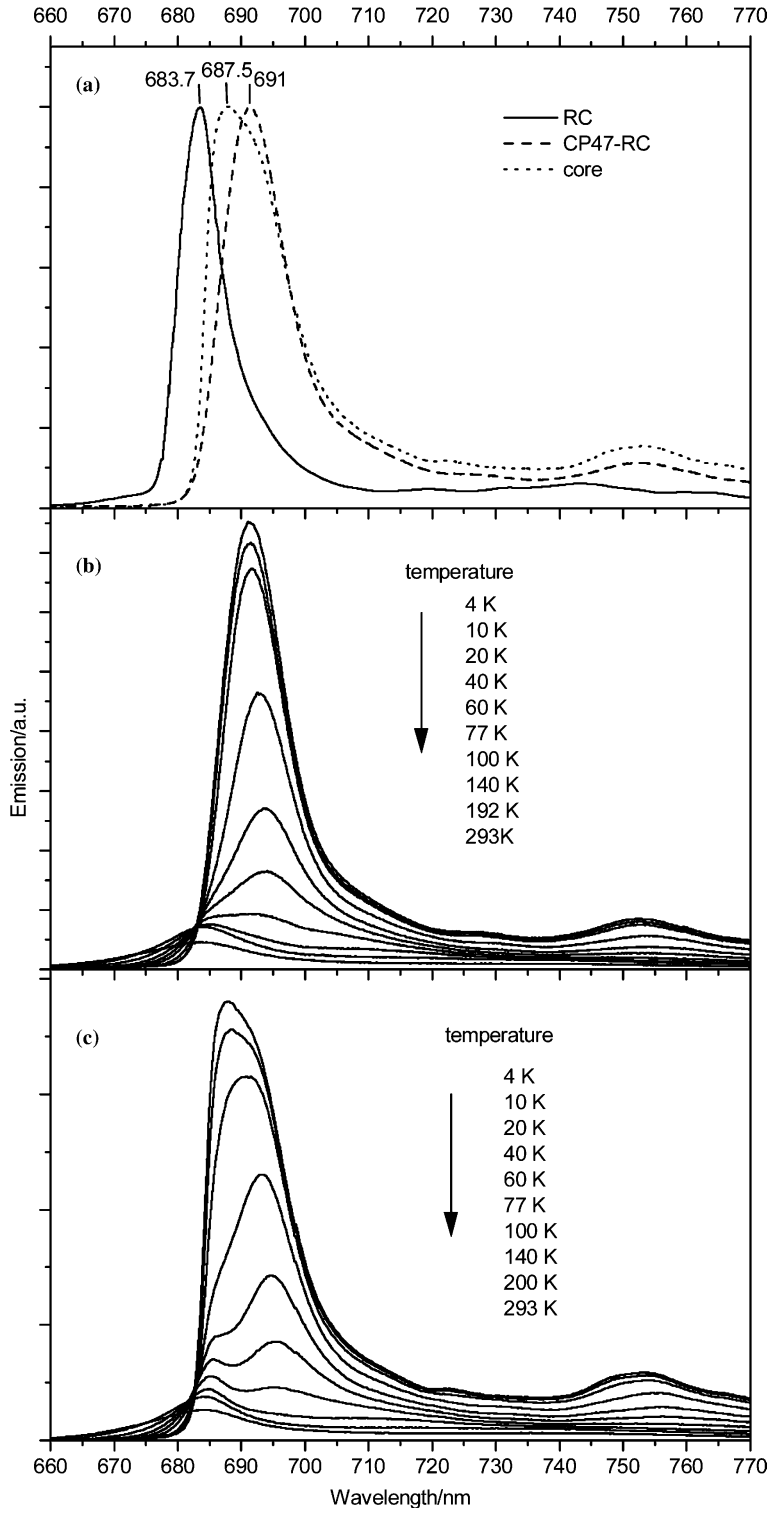

Figure 1. The 4 K emission spectra of the RC (reproduced from Peterman et al. 1998), CP47-RC and PS II core (a); temperature-dependent emission of the CP47-RC (b) and PS II core (c).

(Figure 1c) to 694 and 695 nm, respectively, which is accompanied by an at least four-fold decrease of the emission yield. The 687.5 nm peak in the 4 K emission spectrum of the PS II core complex diminishes strongly at higher temperatures, and at 40 K only a shoulder is observed at about 687 nm next to a band peaking at 693 nm. Around 77 K, a new band appears at 685 nm, which is more clearly resolved in the PS II core complex than in CP47-RC. Thus, the 77 K emission spectrum of the PS II core complex emission shows the well-known F-685 and F-695 emission bands. A further increase of the temperature from 77 K to room temperature results in both complexes in the gradual disappearance of the F-695 emission, and in a blue shift of the F-685 emission to 683 nm together with a further decrease in intensity.

Figure 2 shows the temperature dependence of the relative fluorescence quantum yields of the CP47-RC and core complexes. Both complexes reveal very similar temperature dependences. The quantum yield of CP47-RC and core emission drops about 10 times upon a temperature increase from 4 to 293 K, while that of the isolated RC, CP47 and CP43 complexes drops by a factor of about two in the same temperature range (Groot et al. 1994, 1995, 1999). We note that very similar temperature dependencies of steady-state emission spectra are reported for PS II membrane and core complexes from spinach (Krausz et al. 2005).

The relative contribution of CP43 and CP47 to the absorbance of the PS II core is different at different wavelengths. Figure 3 shows the absorbance spectrum of the PS II core complex and the isolated CP43 and CP47 complexes at 4 K. It is clear from the figure that CP47 dominates the absorbance in the range 675–700 nm and that excitation in the different ranges of absorption of the PS II core populates singlet states of CP43 and CP47 in different proportions.

We performed site-selective emission studies on PS II core complexes to further elucidate the origin of the core emission at 4 K. The sample was excited in the range of 657–695 nm. Figure 4a shows that the shape of the 4 K emission spectrum differs slightly if the sample is excited at 669 nm or at 681 nm. Although both excitation wavelengths excite both CP47 and CP43, the 669 nm light will excite CP43 to a slightly larger extent, while the 681 nm light will preferentially excite CP47 (Figure 3). This result indicates that the additional 687 nm emission in the core complex arises from CP43. This is confirmed by the results presented in Figure 4b, where we plot the relative contribution to the 687 nm emission. It turns out that the amplitude of this emission in the normalized spectra changes with excitation wavelength as expected for isolated CP43.

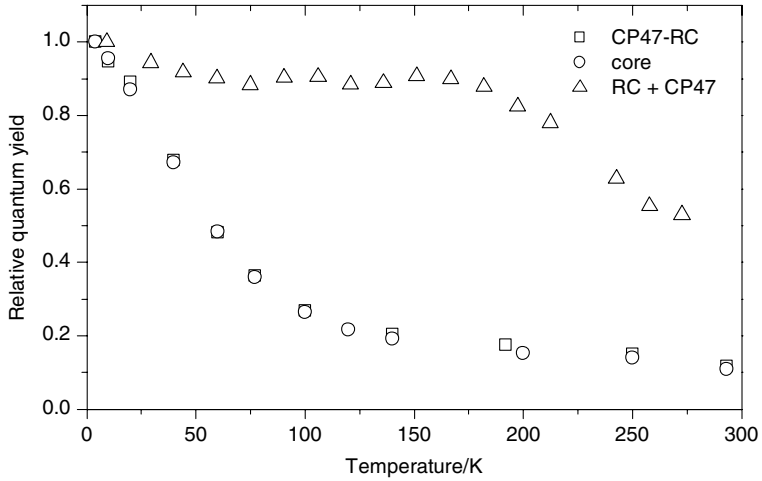

Figure 2. Relative quantum yields of emission for CP47-RC and PS II core in comparison with normalized at 4 K sum of absolute quantum yields for RC and CP47. The absolute quantum yields for RC and CP47 were taken from Groot et al. 1994 and Groot et al. 1995, respectively.

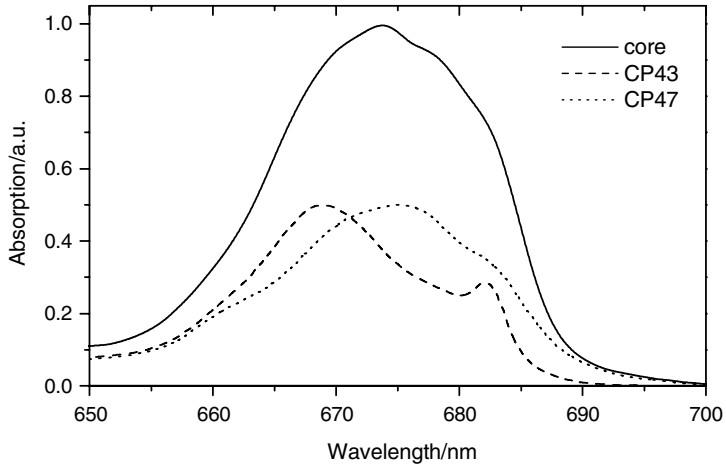

Figure 3. Absorption spectra of the PS II core, CP43 and CP47 at 4 K. The spectra from CP43 and CP47 were taken Groot et al. 1999 and Groot et al. 1995, respectively.

Discussion

There are several factors that contribute to the temperature dependence of the fluorescence yield. The first is a decrease of the yield by a factor of about two upon a temperature increase from 4 to 293 K. This decrease was observed in isolated RC, CP47 and CP43 complexes (Groot et al. 1994, 1995, 1999) and explained by an increase of the internal conversion rate with increasing temperature (Groot et al. 1995). This effect is also expected to play a role in the CP47-RC and PS II core complexes.

For explaining the remaining part of the temperature dependence we refer to the simplified scheme of energy transfer and charge separation in Figure 5. This scheme is based on our fluorescence lifetime studies of the CP47-RC complex (Andrizhiyevskaya et al. 2004). For the sake of simplicity, the peripheral chlorophylls of the RC were omitted (because they play a very minor role in the energy transfer processes within the CP47-RC and larger PSII complexes), while the energy level of the core antenna was broadened, to include CP43 and several energetically different pigment pools in the core antenna chlorophylls. Figure 6 shows that the integrated fluorescence of PSII, modelled as in Figure 5, is expected to decrease with decreasing temperature, except when the core antenna contains chlorophylls with lower energy than those of the RC. In the case that the average energy level of the core antenna is 2 nm more to the red compared to that of the RC, the yield goes down first, but there appears a steep rise of the emission upon cooling below about 50 K.

The decrease with decreasing temperature originates from the reversible radical pairs in this model. The rate of the uphill back reactions from RP2 and RP3 will slow down with decreasing temperature. So, at lower temperatures the RC will be a better trap for excitation energy than at higher temperatures. The decrease is not observed in our experimental data of the CP47-RC and PS II core complexes (Figure 1), but the rather moderate temperature dependence of the fluorescence quantum yield of both complexes between 300 and 150 K can perhaps be explained by a combination of a decrease with decreasing temperature because of radical pair stabilization, and an increase because of a decreased internal conversion rate with decreasing temperature (see above), and possibly because of other effects (see below) A decrease of the fluorescence yield upon cooling from about 180 to 80 K was observed in isolated PS II RC particles (Groot et al 1994). Other effects that could give rise to an increases of PSII fluorescence with decrease temperature are shrinking of the sample upon cooling (Palacios et al. 2002) and a possible closure of the RC (reducing of Q_A) in PSII core only.

At room temperature, the energy transfer between CP47 and the RC was concluded not to be rate limiting for charge separation (Andrizhiyevskaya et al. 2004). However, when the core

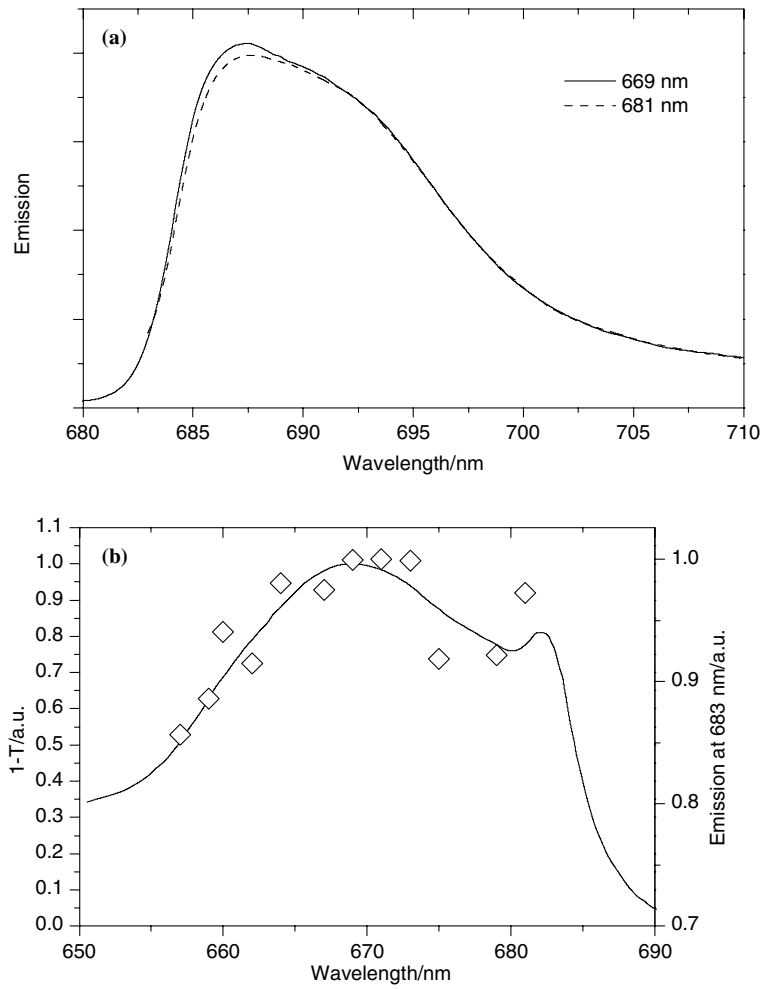

Figure 4. (a) Site-selected emission spectra of the PS II core normalized at 705 nm upon 669 and 681 nm excitations. (b) Emission maxima of these spectra as a function of excitation wavelength compared to the 1-T spectrum of CP43 at 4 K (from Groot et al. 1999). $T = 10^{A}$, where A is absorbance.

antenna contains chlorophylls that absorb at longer wavelength than those of the RC, the uphill energy transfer from these core antenna to the RC will become rate limiting below a certain temperature. This will be accompanied by a slight increase of the fluorescence yield. Because both CP47 and CP43 contain chlorophylls that absorb at slightly lower energies than those of the RC, this suggests that the F-685 fluorescence of PS II represents core emission that is slowly transferred to the RC, where it is largely used for an irreversible charge separation. The energy difference between RP1 and RP2 is probably so large at this temperature that charge separation will be essentially irreversible.

The steep rise of the fluorescence below 50 K arises most likely from trapping of the excitation on the core antenna chlorophylls. If there is not sufficient thermal energy available for the uphill energy transfer to the RC, the excitation will be trapped on the core antenna and decay with its natural fluorescence lifetime. In real pigment–protein complexes the rise will be not as steep as shown in Figure 6, because there are several core antenna chlorophylls that absorb at longer (but slightly different) wavelengths than those of the RC and also because all chlorophylls are inhomogeneously broadened, i.e., absorb at slightly different wavelengths in each individual PS II complex. The inhomogeneous broadening of the

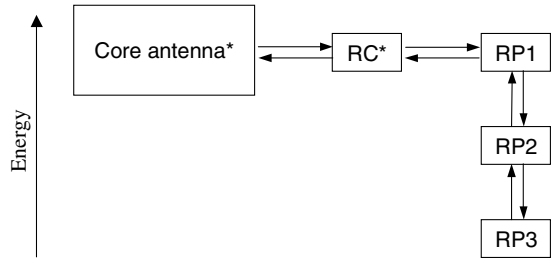

Figure 5. Compartment model of the PS II core complex.

690 nm chlorophyll of CP47 explains the blue shift and the increase in yield of the F-695 fluorescence upon cooling. At 77 K, only for those '690 nm' chlorophylls that absorb at the red side of the inhomogeneous distribution insufficient thermal energy is available for the uphill energy transfer to the RC, so only the red part of the distribution will fluoresce. The blue '690 nm' chlorophylls will still transfer their energy to the RC, which is a non-radiative trap. With decreasing temperature, more and more '690 nm' chlorophylls will not be able to transfer the energy to the RC anymore, resulting in a blue shift and an increase of their fluorescence. It is likely that at 4 K every excitation that ends up on a 690 nm pigment will be trapped on that pigment, because the emission wavelength of isolated CP47 and CP47-RC complexes is the same. The 687 nm fluorescence of CP43 in the PS II core complex, on the other hand, may just represent a minor fraction of CP43 chlorophylls at the red side of the inhomogeneous distribution, because, at least in plants, these absorb maximally at about 682 nm (Groot et al. 1999).

An interesting point here is that the chlorophylls responsible for the irreversible trapping at 77 K do not give rise to detectable triplets (Diner et al., unpublished observations). The triplet is entirely localized on the accessory chlorophyll B_A. This suggests that the 690 nm chlorophylls must be located close to carotenoids that rapidly quench their triplet states, in agreement with previous results on isolated CP47 complexes (Groot et al. 1995).

We conclude that the 77 K F-695 fluorescence originates from excitations that are irreversibly trapped on red-absorbing '690 nm' chlorophylls of CP47 and that the 77 K F-685 fluorescence originates from excitations that are slowly transferred from 683 nm states in both CP47 and CP43 to the RC, where they are irreversibly trapped by charge separation. This transfer will be temperature dependent, and we will address this issue in future studies. At 4 K almost all excitations that reach the '690 nm' chlorophylls of CP47 will be irreversibly trapped on this chlorophyll, whereas some excitations will become trapped on the red-most chlorophylls of a minor fraction of CP43. This interpretation implies that at 4 K a considerable part of the absorbed energy can not be used for charge separation.

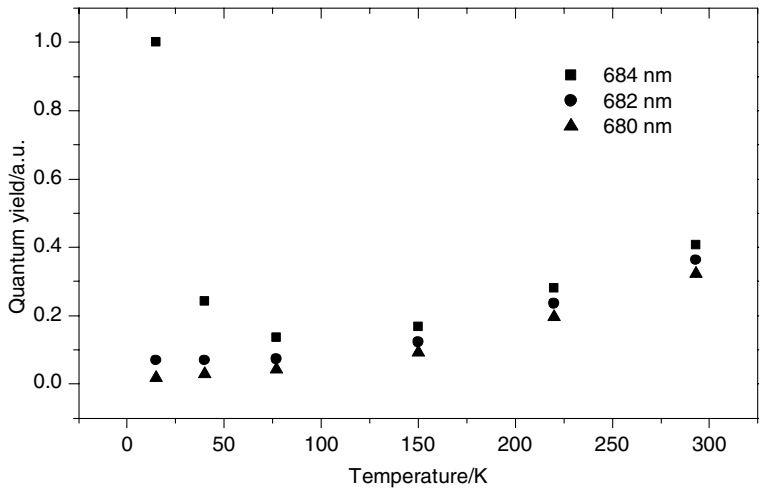

Figure 6. Relative quantum yield of fluorescence, calculated according to the model in Figure 5, with energy levels of core antenna* at 2 nm higher (triangles), the same (circles) and 2 nm lower (squares) levels than that of RC*.

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