ELSEVIER
Biochimica et Biophysica Acta 1555 (2002) 60–64
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

Supramolecular organisation of the photosynthetic chain in anoxygenic bacteria

André Verméglio [a,*], Pierre Joliot [b]

[a] *CEA/Cadarache DEVM-Laboratoire de Bioénergétique Cellulaire, UMR 163-CNRS-CEA, Univ-Méditerranée CEA1000, 13108 Saint Paul lez Durance Cedex, France*
[b] *Institut de Biologie Physico-Chimique, CNRS UPR 9072, 13 rue Pierre et Marie Curie 75005, Paris, France*

Received 12 February 2002; accepted 21 February 2002

Abstract

This minireview summarizes our present view of the supramolecular organization of the photosynthetic apparatus of *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*. These two species present a close association between two reaction centers (RCs), one cytochrome (cyt) bc_1 and one cyt *c*. In *R. sphaeroides*, the RCs are only partially surrounded by LH1 complexes. This open ring of LH1 complexes is required for an efficient photoinduced cyclic electron transfer only under conditions where the quinone pool totally reduced. When the quinone pool is partially oxidized, a closed ring of LH1 complexes around the RCs does not impair the exchange of quinone molecules between the RC and the cyt bc_1 complex. To explain the efficient photochemistry of the various species which possess a RC surrounded by a closed ring of LH, it is proposed that their quinone pool is partially oxidized even under anaerobic condition.

Keywords: Bacterial photosynthesis; Electron transfer; Supercomplex; Light-harvesting complex; Reaction center

1. Introduction

Certain anoxygenic photosynthetic bacteria are among the most versatile microorganisms. Indeed species, like *Rhodobacter sphaeroides* or *Rhodobacter capsulatus*, can grow at the expense of light energy or by developing aerobic or anaerobic respiratory chains. These respiratory chains use oxygen or various organic (TMAO, DMSO) and mineral compounds (nitrate) as electron acceptors [1]. The components involved in these electron transfer chains are localized in the intracytoplasmic membrane or the periplasmic space. Several electron carriers, the cytochrome (cyt) bc_1, the ubiquinone molecules and the cyt c_2, are engaged in both photosynthetic and respiratory processes [1,2]. Although several line of evidence have demonstrated a direct interaction between the photosynthetic and respiratory chains at the cyt c_2 and the ubiquinone level [3–5], biochemical, functional and structural data indicate a high degree of organization of these electron transfer chains. The purpose of the minireview is to summarize our present view of the supramolecular organization of the photosynthetic apparatus in purple bacteria and its possible functional implications.

2. Functional approaches

The presence of a supramolecular organisation of the photosynthetic apparatus was already implicit in the concept of the photosynthetic unit developed by Gaffron and Vohl [6] after the pioneering work of Emerson and Arnold in 1932 [7]. In this concept, antennae pigments (or light-harvesting complexes, LHC) efficiently transfer the excitonic energy to a specialized reaction center (RC) where the photochemistry occurs. The high efficiency of this energy transfer (>90%) necessitates close proximity (50 Å) of the LHC pigments and the chromophores of the RCs. After energy transfer to the RC, a charge separation occurs at its level between the primary electron donor, a bacteriochlorophyll molecule, and the primary acceptor, an ubiquinone molecule. This first photochemical reaction is followed by a cyclic electron transfer where the RC and the cyt bc_1 complex are connected by ubiquinone in the lipid phase and a cyt *c* or a high potential iron–sulfur protein (HiPIP) in the aqueous phase [8–11]. Due to the solubility and

* Corresponding author. Tel.: +33-42-25-4630; fax: +33-44-22-54701.
E-mail address: avermeglio@cea.fr (A. Verméglio).

0005-2728/02/$ - see front matter
PII: S0005-2728(02)00255-4

mobility of these electron carriers, it is generally assumed that the connection between the RC and the cyt bc_1 complex is mediated by random collisions and does not require a particular arrangement for effective electron transfer. However, this view has been challenged in the past few years.

In the case of *R. sphaeroides*, we observed that the apparent equilibrium constant between cyt c_2, cyt bc_1 and the RC is much less than expected from their mid-point potentials [12]. This behavior can be explained by supposing that a rapid thermodynamic equilibrium is achieved at a local level within a domain, or supercomplex, containing a small number of electron carriers, whereas equilibration at a macrolevel between these domains is a much slower process [13]. Simulation of the data suggests that each supercomplex contains two RCs, one cyt c_2 and one cyt bc_1 complex [12]. This implies that the diffusion of cyt c_2 is confined to a small domain including only two RCs and one cyt bc_1. Another argument in favor of the supramolecular organization of the photosynthetic chain comes from the observation that addition of sub-saturating concentration of myxothiazol, a specific inhibitor of the cyt bc_1 complex, decreases the number of active complexes but does not affect the rate of electron transfer for the uninhibited complexes [14]. This indicates that each photosynthetic chain acts as an isolated entity. The necessary proximity between RC, cyt c_2 and cyt bc_1 is also indicated by the observation that the complete cyclic photoinduced electron transfer occurs at -20 °C in frozen medium [15].

In the case of *R. capsulatus*, genetic and biological means have demonstrated the presence of an alternative cytochrome, called c_y, in addition to the cyt c_2 [16,17]. This cyt connects the cyt bc_1 complex to the RC. Hydropathy analyses of its primary structure and biochemical characterization of the chromatophores content in cyt lead to the conclusion that cyt c_y is membrane bound [17]. We have shown by a series of functional approaches that the movement of cyt c_y is restricted to a small membrane domain which includes two RCs and one cyt bc_1 complex [18]. This implies, like for *R. sphaeroides*, a specific arrangement between these different membrane-bound complexes.

3. Structural approaches

Besides these thermodynamic and kinetic arguments, supramolecular organization of the photosynthetic components have been obtained by biochemical and biophysical approaches.

It is only 50 years after the proposal of Emerson and Arnold that a picture of the structure of the photosynthetic unit at low resolution was provided by Miller [19]. This author took advantage of the presence of a regular pattern for the photosynthetic unit in the internal membrane of the purple non-sulfur bacterium *Rhodopseudomonas viridis*. Fourier image analysis of negatively stained membranes of *R. viridis* clearly shows that each photosynthetic unit is composed of a large central structure, the RC center, surrounded by a circle of smaller complexes, the light-harvesting complexes 1 (LH1). This type of organization has been also observed for native membranes or 2D crystals of RC–LH1 complexes of several other species of bacteria (*Ectothiorhodospira halochloris* [20], *Rhodopseudomonas molischianum* [21], *Rhodospirillum rubrum* [22] and *R. sphaeroides* [23]). While this association is consistent with an efficient and rapid energy transfer between LHC and RC, the closed structure of LH1 raised the question of how the quinol formed at the acceptor site of the RC could reach the cyt bc_1 complex to complete the light-induced cyclic electron transfer.

A partial answer was obtained, in the case of *R. sphaeroides*, by the analysis of freeze-fracture electron microscopy of tubular membranes [24,25]. They reveal a well-ordered arrangement of dimeric particles of 110 Å in diameter (Fig. 1A). These tubular membranes correspond to regions of the intracytoplasmic membrane devoid of LH2 complexes obtained by gene deletion or growth under semi-aerobic conditions [24–26]. They contain all the components of the photosynthetic chain, RC, LH1 and cyt bc_1 complex. Due to the well-ordered arrangement of the particles, electron micrograph of negatively stained samples diffract at 20 Å

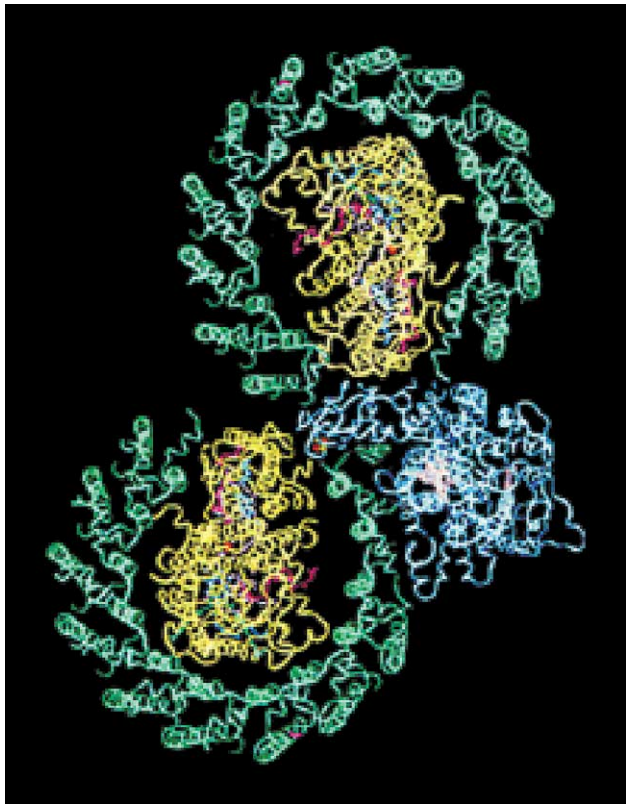

Fig. 1. Schematic representation of the supramolecular organization of the photosynthetic apparatus of *R. sphaeroides* view from above the membrane. The LH1 complexes are in green, the RCs in yellow and the cyt bc_1 complex in blue. This has been deduced from the projection map at 20 Å resolution of negatively stained tubular membranes [27].

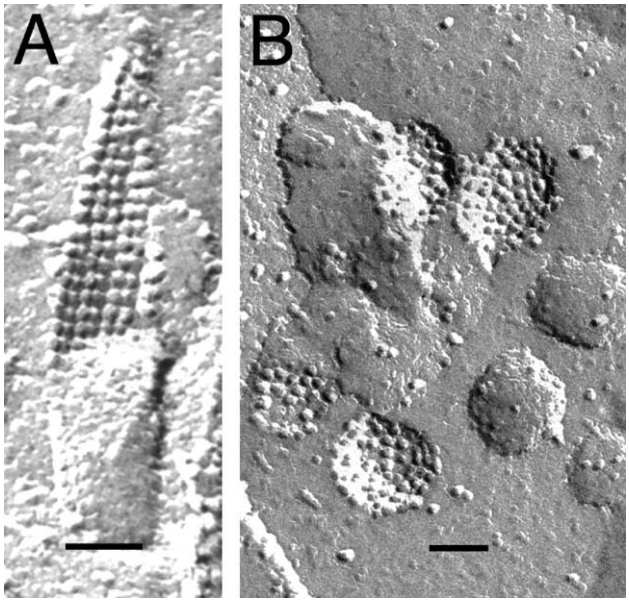

Fig. 2. Freeze-etching pictures of the intracytoplasmic membranes present in *R. sphaeroides* WT (A) and mutant deleted in pufX (B). Scale bar = 50 nm.

and allows the determination of the supramolecular organization of the photosynthetic apparatus [27]. Analysis of the positive density from the proteins indicates that the unit cell contains two RCs partially surrounded by LH1 complexes. A schematic representation of the supramolecular arrangement of the photosynthetic apparatus based on the proteins density, the composition in membrane complexes and the 3D structure of these individual complexes is depicted in Fig. 2. This highlights not only the supramolecular organization of the photosynthetic apparatus in agreement with the functional approaches, in particular the dimeric association of the RCs, but also that these RCs are surrounded by an open ring of the LH1. The open ring of LH1 around RC observed in the tubular membranes of *R. sphaeroides* would facilitate diffusion of quinone molecules between the RC and the cyt bc_1 complex.

4. Open or closed ring?

The dimeric association of RCs in *R. sphaeroides* has also been demonstrated by Francia et al. [28] using a biochemical approach. After detergent solubilization of chromatophores, these authors found two membrane complexes corresponding to monomeric and dimeric RC–LH1 complexes in addition to isolated LH1 and LH2 complexes [28]. The dimeric RC–LH1 complexes comprise two intertwined rings of LH1 containing two RCs in agreement with the organization shown in Fig. 1. The Bchl/RC ratio of these dimeric structures is significantly lower than that measured for the monomeric RC–LH1 complexes. This is also consistent with an open ring of LH1 in the dimeric complexes [28].

These authors have also shown that the PufX polypeptide is strictly required for the isolation of dimeric RC–LH1 complexes. This polypeptide is encoded by the *pufX* gene, localized in the puf (photosynthetic formation unit) operon downstream of the genes encoding the LH1 and L and M subunits of the RC. So far, this gene has only been found in *R. sphaeroides* and *R. capsulatus* [29,30]. The PufX polypeptide is a membrane protein closely associated with the RC–LH1 complex in a 1:1 ratio [28]. The presence of the polypeptide is essential for anaerobic photosynthetic growth in both *R. capsulatus* and *R. sphaeroides* [29, 30]. Since the pufX polypeptide inhibits the in vitro oligomerization of LH1 complexes, it is tempting to speculate that this protein may play an essential role in the formation of the open LH1 structure observed in vivo [31]. In the absence of pufX, a complete ring of LH1 may be formed around the RC. This is

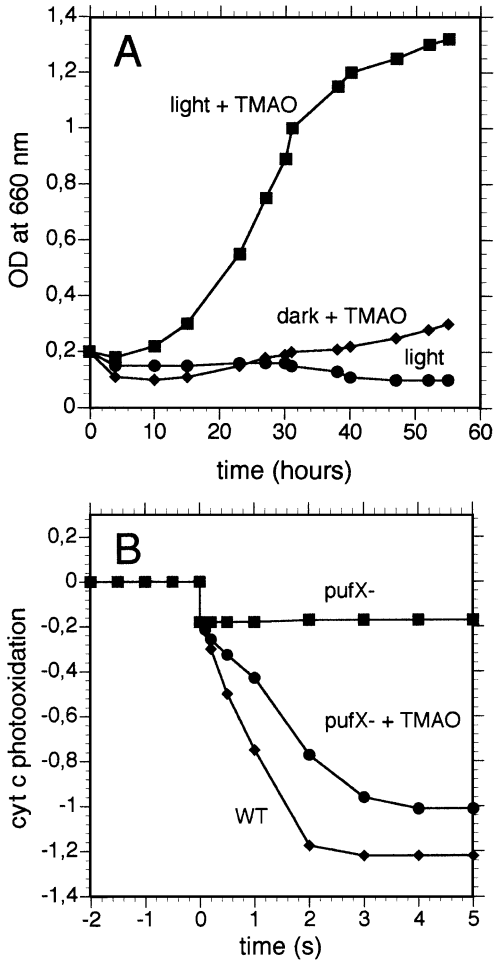

Fig. 3. (A) Growth curves of the mutant of *R. sphaeroides* deleted in the gene *pufX* for various growth conditions: in light (●), in light in the presence of 20 mM TMAO (■) and in the dark in the presence of 20 mM TMAO (♦). (B) Kinetics of cytochrome *c* photooxidation following a continuous illumination (started at time 0) for intact cells of *R. sphaeroides* (WT, ♦) and for the mutant deleted in the gene *pufX* (pufX-). The cells of the mutant were placed under anaerobic condition in the presence (●) or the absence (■) of 20 mM TMAO.

in agreement with the observation that deletion of pufX induces a significant increase in the bacteriochlorophyll molecules to RCs (i.e. LH1/RC) [32,33] and that crystals of RC–LH1 particles isolated from the pufX- mutant present a complete ring around the RC [23]. The presence of a complete ring of LH1 around the RC in the mutant deleted in pufX could inhibit the diffusion of quinone and therefore the connection between the RC and the cyt bc_1 complex. This can readily explain the inability of this mutant to grow under phototrophic anaerobic condition (Fig. 3). It is, however, important to note that photosynthetic growth and the light-induced cyclic electron transfer are restored in the pufX- mutant when the quinone pool is partially oxidized by the addition of an electron acceptor like the TMAO or the DMSO [34]. The effect of addition of TMAO on the growth curves and the cyt photooxidation of the mutant deleted in the pufX polypeptide is shown in Fig. 3. Another effect of the deletion of pufX polypeptide is the disappearance of the tubular membranes. This emphasizes the important role of this polypeptide in the supramolecular organisation of the photosynthetic apparatus (Fig. 1B). This observation is in agreement with the report of Frese et al. [35]. These authors have shown by linear dichroism measurements on oriented membranes that the polypeptide pufX is required for the formation of a long-range regular array of supramolecular photosynthetic units [35].

These results imply that neither the supramolecular organization of the photosynthetic apparatus nor the open structure of the LH1 in *R. sphaeroides* are necessary for an efficient cyclic electron transfer. In the case of *R. sphaeroides* and *R. capsulatus*, the open ring of LH1 appears to be necessary only when the quinone pool is totally reduced.

Why the necessity of a supramolecular organisation of the photosynthetic apparatus in *R. sphaeroides* and *R. capsulatus* when the quinone pool is fully reduced? Under such redox conditions, the only oxidized quinone molecules are formed at the level of the cyt bc_1 after charge separation. The concentration in oxidized quinone is very low (1 quinone for about 50 quinol molecules). Due to stoichiometry between RCs and cyt bc_1 (two RCs/one cyt bc_1), this last complex has to make two distinct turnovers to reduce the photooxidized cyt c_2. After the first turnover of the cyt bc_1 complex, the quinone formed at the periplasmic side has to be reduced at the cytoplasmic side of this complex to allow its second turnover. This second turnover will produce a second oxidized quinone molecule which can exchange with one of the reduced molecule formed at the reducing site of the RCs. The close proximity of the RCs and the cyt bc_1 in the supercomplex will render this exchange very efficient and allow further charge separation at the level of the RC. In other terms, an efficient electron transfer between the RC and the cyt bc_1 is possible despite the low concentration of oxidized quinones because of their confinement in the supercomplex.

On the other hand, it is possible that for the various species, where it has been shown that RCs are surrounded by a closed ring of LH1 in native membranes, physiological conditions are such that, even under anaerobic condition, the quinone pool is partially oxidized. These can be achieved by several means. For example, the quinone pool can be maintain partially oxidized due to the presence of auxiliary electron acceptor chain. Another possibility is that there is no thermodynamic equilibration at the quinone pool level between the photosynthetic and respiratory chains. This is probably the case for species like *R. viridis* or *Thiocapsa pfennigii* (Fig. 4) where the high ordering of the photosynthetic apparatus in the intracytoplasmic membrane certainly renders difficult the long-range diffusion of quinone molecules.

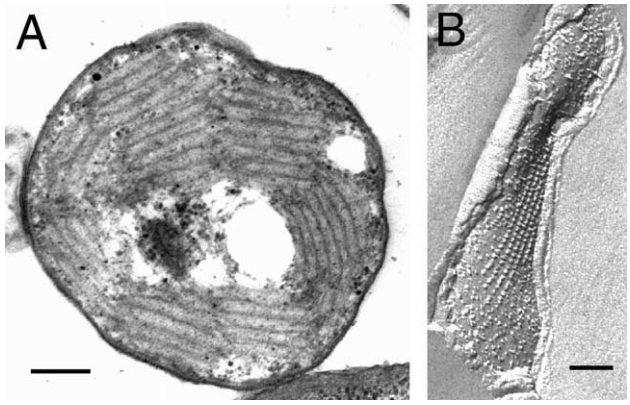

Fig. 4. (A) Thin-section electron micrograph of *T. pfennigii* showing the tubular shape of the intracytoplasmic membrane. Scale bar = 300 nm. (B) Freeze-etching picture of the tubular membrane underlining the arrangement of the photosynthetic unit. Scale bar = 100 nm.

Acknowledgements

We wish to thank Wolfgang Barz, Anne Joliot, Colette Jungas, Jerôme Lavergne, Dieter Oesterhelt, Jacqueline Olive, Jean-Luc Ranck, Jean-Louis Rigaud and Monique Sabaty for their collaborative work in the study of the photosynthetic apparatus of anoxygenic bacteria.

References

[1] S.T. Ferguson, J.B. Jackson, A.G. Mc Ewan, FEMS Microbiol. Rev. 46 (1987) 117–143.
[2] J.B. Jackson, Electron transfer in photosynthetic bacteria, in: C. Anthony (Ed.), Bacterial Energy Transduction, Academic Press, 1988, pp. 317–375.
[3] D. Zannoni, P. Jasper, B.L. Marrs, Biochim. Biophys. Acta 191 (1978) 625–631.
[4] A. Verméglio, J.M. Carrier, Biochim. Biophys. Acta 764 (1984) 233–238.
[5] M. Sabaty, P. Gans, A. Verméglio, Arch. Microbiol. 159 (1993) 153–159.
[6] H. Gaffron, K. Vohl, Natur Wiesen Schasten 24 (1936) 81–103.
[7] R. Emerson, W. Arnold, J. Gen. Physiol. 16 (1932) 191–205.
[8] A.R. Crofts, C.A. Wraight, Biochim. Biophys. Acta 726 (1983) 149–185.

[9] T.E. Meyer, T.J. Donohue, Cytochromes, iron-sulfur, and copper proteins mediating electron transfer from the cyt bc_1 complex to photosynthetic reaction center complexes, in: R.E. Blankenship, M.T. Madigan, C.E. Bauer (Eds.), Anoxygenic Photosynthetic Bacteria, Kluwer Academic, Dordrecht, 1995, pp. 775–805.
[10] B. Schoepp, P. Parot, L. Menin, J. Gaillard, P. Richaud, A. Verméglio, Biochemistry 34 (1995) 11736–11742.
[11] A. Hochkoeppler, S. Ciurli, G. Venturoli, D. Zannoni, FEBS Lett. 357 (1995) 70–74.
[12] P. Joliot, A. Verméglio, A. Joliot, Biochim. Biophys. Acta 975 (1989) 336–345.
[13] J. Lavergne, P. Joliot, A. Verméglio, Biochim. Biophys. Acta 975 (1989) 346–354.
[14] P. Joliot, A. Verméglio, A. Joliot, Photosynth. Res. 48 (1996) 291–299.
[15] P. Joliot, A. Joliot, A. Verméglio, Biochim. Biophys. Acta 1318 (1997) 374–388.
[16] M.R. Jones, A.G. McEwan, J.B. Jackson, Biochim. Biophys. Acta 1019 (1990) 59–66.
[17] F.E. Jenney, F. Daldal, EMBO J. 12 (1993) 1283–1292.
[18] A. Verméglio, A. Joliot, P. Joliot, Biochim. Biophys. Acta 56 (1998) 329–337.
[19] K.R. Miller, Nature 300 (1982) 53–55.
[20] W. Stark, W. Kühlbrandt, J. Wildhaber, E. Wehrli, K. Mühlethaler, EMBO J. 3 (1984) 777–783.
[21] A.F. Boonstra, L. Germeroth, E.J. Boekema, Biochim. Biophys. Acta 1184 (1994) 227–234.
[22] T. Walz, R. Ghosh, J. Mol. Biol. 265 (1997) 107–111.
[23] T. Walz, S.J. Jamieson, C.M. Bowers, P.A. Bullough, C.N. Hunter, J. Mol. Biol. 282 (1998) 833–845.
[24] C.N. Hunter, J.D. Pennoyer, J.N. Sturgis, D. Farrely, R.A. Niederman, Biochemistry 27 (1988) 3459–3467.
[25] P.J. Kiley, A. Varega, S. Kaplan, J. Bacteriol. 170 (1988) 1103–1115.
[26] M. Sabaty, J. Jappé, J. Olive, A. Verméglio, Biochim. Biophys. Acta 1187 (1994) 313–323.
[27] C. Jungas, J.L. Ranck, J.L. Rigaud, P. Joliot, A. Verméglio, EMBO J. 18 (1999) 534–542.
[28] F. Francia, J. Wang, G. Venturoli, B.A. Melandri, W.P. Barz, D. Oesterhelt, Biochemistry 38 (1999) 6834–6845.
[29] J.M. Farchaus, H. Grünberg, D. Oesterhelt, J. Bacteriol. 172 (1990) 977–985.
[30] T.G. Liburn, C.E. Haith, R.C. Prince, J.T. Beatty, J. Biochim. Biophys. Acta 1100 (1992) 160–170.
[31] P.A. Recchia, C.M. Davis, T.G. Lilburn, J.T. Beatty, P.S. Parkes-Loach, C.N. Hunter, P.A. Loach, Biochemistry 37 (1998) 11055–11063.
[32] P. McGlynn, H.J. Willem, H.T. Westerhuis, M.R. Jones, C.N. Hunter, J. Biol. Chem. 271 (1996) 3285–3292.
[33] W.H.J. Westerhuis, J.H. Farchaus, R.A. Niederman, Photochem. Photobiol. 58 (1993) 460–463.
[34] W.P. Barz, A. Verméglio, F. Francia, G. Venturoli, B.A. Melandri, D. Oesterhelt, Biochemistry 34 (1995) 15248–15258.
[35] R.N. Frese, J.D. Olsen, R. Brandall, W.H.J. Westerhuis, C.N. Hunter, R. van Grondelle, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 5197–5202.