

# Photosynthetic Light-Harvesting Systems Organization and Function

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Hugo Scheer · Siegfried Schneider



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## CONTENTS

List of Participants .....	XIII
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### SECTION I. ORGANIZATION: BIOCHEMICAL METHODS

Introduction: The Biochemistry of Light-Harvesting Complexes by R.J. Cogdell .....	1
Phycobilisome-Thylakoid Interaction: The Nature of High Molecular Weight Polypeptides by E. Gantt C.A. Lipschultz and F.X. Cunningham Jr. ....	11
On the Structure of Photosystem II-Phycobilisome Complexes of Cyanobacteria by E. Mörschel and G.-H. Schatz .....	21
Structure of Cryptophyte Photosynthetic Membranes by W. Wehrmeyer .....	35
Structural and Phylogenetic Relationships of Phycoerythrins from Cyanobacteria, Red Algae and Cryptophyceae by W. Sidler and H. Zuber .....	49
Isolation and Characterization of the Components of the Phycobilisome from <u>Mastigocladus laminosus</u> and Cross- linking Experiments by R. Rübeli and H. Zuber .....	61
C-Phycocyanin from <u>Mastigocladus laminosus</u> : Chromophore Assignment in Higher Aggregates by Cystein Modification by R. Fischer, S. Siebzehrübl and H. Scheer .....	71
Photochromic Properties of C-Phycocyanin by G. Schmidt, S. Siebzehrübl, R. Fischer and H. Scheer .....	77
Concerning the Relationship of Light Harvesting Bili- proteins to Phycochromes in Cyanobacteria by W. Kufer .....	89
Subunit Structure and Reassembly of the Light-Harvesting Complex from <u>Rhodospirillum rubrum G9+</u> by R. Ghosh, Th. Rosatzin and R. Bachofen .....	93
Primary Structure Analyses of Bacterial Antenna Polypeptides - Correlation of Aromatic Amino Acids with Spectral Properties - Structural Similarities with Reaction Center Polypeptides by R.A. Brunisholz and H. Zuber .....	103

The Structure of the "Core" of the Purple Bacterial Photo-synthetic Unit by D.J. Dawkins, L.A. Ferguson and R.J. Cogdell .....	115
A Comparison of the Bacteriochlorophyll C--Binding Proteins of Chlorobium and Chloroflexus by P.D. Gerola, P. Højrup and J.M. Olson .....	129
Interactions between Bacteriochlorophyll c Molecules in Oligomers and in Chlorosomes of Green Photosynthetic Bacteria by D.C. Brune, G.H. King and R.E. Blankenship .....	141
Light-Harvesting Complexes of Chlorophyll c-Containing Algae by A.W.D. Larkum and R.G. Hiller .....	153
Isolation and Characterization of a Chlorophyll a/c-Hetero-xanthin/Diadinoxanthin Light-Harvesting Complex from <i>Pleurochloris meiringensis</i> (Xanthophyceae) by C. Wilhelm, C. Büchel and B. Rousseau .....	167
The Antenna Components of Photosystem II with Emphasis on the Major Pigment-Protein, LHC IIb by G.F. Peter and P. Thornber .....	175

## SECTION II: ORGANIZATION: MOLECULAR GENETICS AND CRYSTALLOGRAPHY

Molecular Biology of Antennas by G. Drews .....	187
High-Resolution Crystal Structure of C-Phycocyanin and Polarized Optical Spectra of Single Crystals by T. Schirmer, W. Bode and R. Huber .....	195
Crystallization and Spectroscopic Investigation of Purple Bacterial B800-850 and RC-B875 Complexes by W. Welte, T. Wacker and A. Becker .....	201
Structure of the Light-Harvesting Chlorophyll a/b-Protein Complex from Chloroplast Membranes by W. Kühlbrandt .....	211
Phycobilisomes of <i>Synchococcus</i> Sp. PCC 7002, <i>Pseudanabaena</i> Sp. PCC 7409, and <i>Cyanophora paradoxa</i> : An Analysis by Molecular Genetics by D.A. Bryant .....	217
Organization and Assembly of Bacterial Antenna Complexes by G. Drews .....	233

The Use of Mutants to Investigate the Organization of the Photosynthetic Apparatus of <u>Rhodobacter sphaeroides</u> by C.N. Hunter and R. van Grondelle .....	247
Mechanisms of Plastid and Nuclear Gene Expression During Thylakoid Membrane Biogenesis in Higher Plants by P. Westhoff, H. Grüne, H. Schrubar, A. Oswald, M. Streubel, U. Ljungberg and R.G. Herrmann .....	261
SECTION III: ORGANIZATION: SPECIAL SPECTROSCOPY TECHNIQUES AND MODELS	
Assignment of Spectral Forms in the Photosynthetic Antennas to Chemically Defined Chromophores by A. Scherz .....	277
Linear Dichroism and Orientation of Pigments in Phycobilisomes and their Subunits by L. Juszczak, N.E. Geacintov, B.A. Zilinskas and J. Breton .....	281
Low Temperature Spectroscopy of Cyanobacterial Antenna Pigments by W. Köhler, J. Friedrich, R. Fischer and H. Scheer .....	293
Chromophore Conformations in Phycocyanin and Allophycocyanin as Studied by Resonance Raman Spectroscopy by B. Szalontai, V. Csizmadia, Z. Gombos, K. Csatorday and M. Lutz .....	307
Coherent Anti-Stokes Raman Spectroscopy of Phycobilisomes, Phycocyanin and Allophycocyanin from <u>Mastigocladus</u> <u>laminosus</u> by S. Schneider, F. Baumann, W. Steiner, R. Fischer, S. Siebzehrübl and H. Scheer .....	317
Optical Absorption and Circular Dichroism of Bacteriochlorophyll Oligomers in Triton X-100 and in the Light-Harvesting-Complex B850; A Comparative Study by V. Rozenbach-Belkin, P. Braun, P. Kovatch and A.Scherz .....	323
Absorption Detected Magnetic Resonance in Zero Magnetic Field on Antenna Complexes from <u>Rps. acidophila</u> 7050 - The Temperature Dependence of the Carotenoid Triplet State Properties by J. Ullrich, J.U. v. Schütz and H.C. Wolf .....	339
Effect of Lithium Dodecyl Sulfate on B 800-850 Antenna Complexes from <u>Rhodospseudomonas acidophila</u> : A Resonance Raman Study by B. Robert and H. Frank .....	349

Bacteriochlorophyll a/b in Antenna Complexes of Purple Bacteria by B. Robert, A. Vermeglio, R. Steiner, H. Scheer and M. Lutz .....	355
Bacteriochlorophyll c Aggregates in Carbon Tetrachloride as Models for Chlorophyll Organization in Green Photo- synthetic Bacteria by J.M. Olson and J.P. Pedersen .....	365
Orientation of the Pigments in the Reaction Center and the Core Antenna of Photosystem II by J. Breton, J. Durantou and K. Satoh .....	375
Non-Linear Absorption Spectroscopy of Antenna Chlorophyll a in Higher Plants by D. Leupold, H. Stiel and P. Hoffmann .....	387

## SECTION IV: FUNCTION: ELECTRONIC EXCITATION AND ENERGY TRANSFER

Excitation Energy Transfer in Photosynthesis by R. van Grondelle and V. Sundström .....	403
Fluorescence Spectroscopy of Allophycocyanin Complexes from <u>Synechococcus 6301 Strain AN112</u> by P. Maxson, K. Sauer and A.N. Glazer .....	439
Picosecond Energy Transfer Kinetics in Allophycocyanin Aggregates from <u>Mastigocladus laminosus</u> by E. Bittersmann, W. Reuter, W. Wehrmeyer and A.R. Holzwarth .....	451
Picosecond Time-Resolved Energy Transfer Kinetics within C-Phycocyanin and Allophycocyanin Aggregates by T. Gillbro, A. Sandström, V. Sundström, R. Fischer and H. Scheer .....	457
Energy Transfer in "Native" and Chemically Modified C-Phyco- cyanin Trimers and the Constituent Subunits by S. Schneider, P. Geiselhart, F. Baumann, S. Siebzehnrübl, R. Fischer and H. Scheer .....	469
Effect of Protein Environment and Excitonic Coupling on the Excited-State Properties of the Bilinchromophores in C-Phycocyanin by S. Schneider, Ch. Scharnagl, M. Dürring, T. Schirmer and W. Bode .....	483
Excitation Energy Migration in C-Phycocyanin Aggregates Isolated from <u>Phormidium luridum</u> : Predictions from the Förster's Inductive Resonance Theory by J. Grabowski and G.S. Björn .....	491

Energy Transfer Calculations for two C-Phycocyanins Based on Refined X-Ray Crystal Structure Coordinates of Chromophores by K. Sauer and H. Scheer ..... 507

Energy Transfer in Light-Harvesting Antenna of Purple Bacteria Studied by Picosecond Spectroscopy by V. Sundström, H. Bergström, T. Gillbro, R. van Grondelle, W. Westerhuis, R.A. Niederman and R.J. Cogdell ..... 513

Excitation Energy Transfer in the Light-Harvesting Antenna of Photosynthetic Purple Bacteria: The Role of the Long-Wave-Length Absorbing Pigment B896 by R. van Grondelle, H. Bergström, V. Sundström, R.J. van Dorssen, M. Vos and C.N. Hunter ..... 519

The Function of Chlorosomes in Energy Transfer in Green Photosynthetic Bacteria by R.J. van Dorssen, M. Vos and J. Amesz ..... 531

Energy Transfer in *Chloroflexus aurantiacus*: Effects of Temperature and Anaerobic Conditions by B.P. Wittmershaus, D.C. Brune and R.E. Blankenship ..... 543

Interpretation of Optical Spectra of Bacteriochlorophyll Antenna Complexes by R.M. Pearlstein ..... 555

Time Resolution and Kinetics of "F680" at Low Temperatures in Spinach Chloroplasts by R. Knox and S. Lin ..... 567

Picosecond Studies of Fluorescence and Absorbance Changes in Photosystem II Particles from *Synechococcus* Sp. by A.R. Holzwarth, G.H. Schatz and H. Brock ..... 579

Analysis of Excitation Energy Transfer in Thylakoid Membranes by the Time-Resolved Fluorescence Spectra by M. Mimuro ..... 589

V. CONCLUDING REMARKS

Future Problems on Antenna Systems and Summary Remarks by E. Gantt ..... 601

Author Index ..... 605

Subject Index ..... 609

EFFECT OF PROTEIN ENVIRONMENT AND EXCITONIC COUPLING ON  
THE EXCITED-STATE PROPERTIES OF THE BILINCHROMOPHORES IN  
C-PHYCOCYANIN

S. Schneider, Ch.Scharnagl

Institut für Physikalische und Theoretische Chemie der  
Technischen Universität München, D-8046 Garching

M. Duerring, T. Schirmer, W. Bode

Max-Planck-Institut für Biochemie, D-8033 Martinsried

Introduction

C-Phycocyanin (C-PC), one of the light-harvesting pigments of cyanobacteria, is composed of two subunits,  $\alpha$  and  $\beta$ . The former contains one, the latter two open-chain tetrapyrrole chromophores (phycocyanobilin), covalently bound to the apoprotein via thioether linkages (see e.g. (1)).

The geometries and individual environments of the three chromophores in the phycocyanin of Mastigocladus laminosus have recently been determined by peptide sequencing (2) and high resolution X-ray crystallography (3a,b,c). The crystal structure displays a strong similarity of geometries, so that different spectroscopic, biochemical and functional properties must be mainly attributed to the different protein surroundings. Lately it has been possible to assign the absorption bands (maxima) to the different phycocyanin chromophores unambiguously in the following manner (4,5):

A84:	$\lambda_{\max}(\text{abs}) = 616 - 618 \text{ nm}$ (4) ;	618 nm (5)
B84:	622 - 624 nm (4) ;	624 - 632 nm (5)
B155:	598 - 600 nm (4) ;	594 - 598 nm (5)
		582 - 598 nm (5,CD)

CD-spectroscopy is an important source of information to elucidate the conformation of bilin chromophores and a sensitive probe to noncovalent interactions. Therefore we studied the effect of protein-chromophore and chromophore-chromophore interactions on the chiroptic properties by means of quantum mechanical model calculations, in order to improve our knowledge about the contribution of these interactions to the spectral and functional properties of the phycobilinproteins and to get an estimate of the significance of these interactions.

## Methods

Our calculations are based on the high-resolution X-ray data for chromophore and protein (3c). Approximative wavefunctions result from a  $\pi$ -electron model calculation (PPP) with standard parametrisation and configuration interaction (CI) of singly excited states (CI-basis: 6 occupied, 6 virtual molecular orbitals; a larger CI-basis leads to a 10 to 15 nm redshift and small variations in the other calculated properties). The rotatory strengths and f-values are calculated without further approximations (6a,6b,7).

The chromophores and their surroundings are treated as one "supermolecule". Therefore, all interactions between permanent and transition charge distributions are included. Polar and aromatic amino acid residues lying in a sphere with 10 Å radius around C10 were taken into account.

Hydrogen bonds are simulated by varying the effective ionization potential (IP') of the heteroatom since a withdrawal of the hydrogen from a pyrrole nitrogen causes the  $\pi$ -electrons of this center to delocalize more easily. The parameter IP' was changed accordingly from 19.6 eV (no bonding) to 16.5 eV (strong bonding, IP' midway between the values for pyrrole and pyridine nitrogen). The values of all other parameters are given elsewhere (6,7).



## Results and Discussion

### Single Chromophores

Figures 1 and 2 show the variations of the absorption wavelength and rotatory strength R of the red band of B84 and B155, respectively, due to chromophore-protein interactions. We restrict our discussion to this band because it is well separated from other transitions and therefore easier to analyse - theoretically as well as experimentally.

Case I: isolated chromophore, neglecting all side chains

Case II: I + propionic acid side chains attached to C8 + C12

Case III: II + aspartate residue near pyrrole rings B and C  
(B87 and B39, respectively)

Case IV: III + residues of polar amino acids near the  
propionic side chains

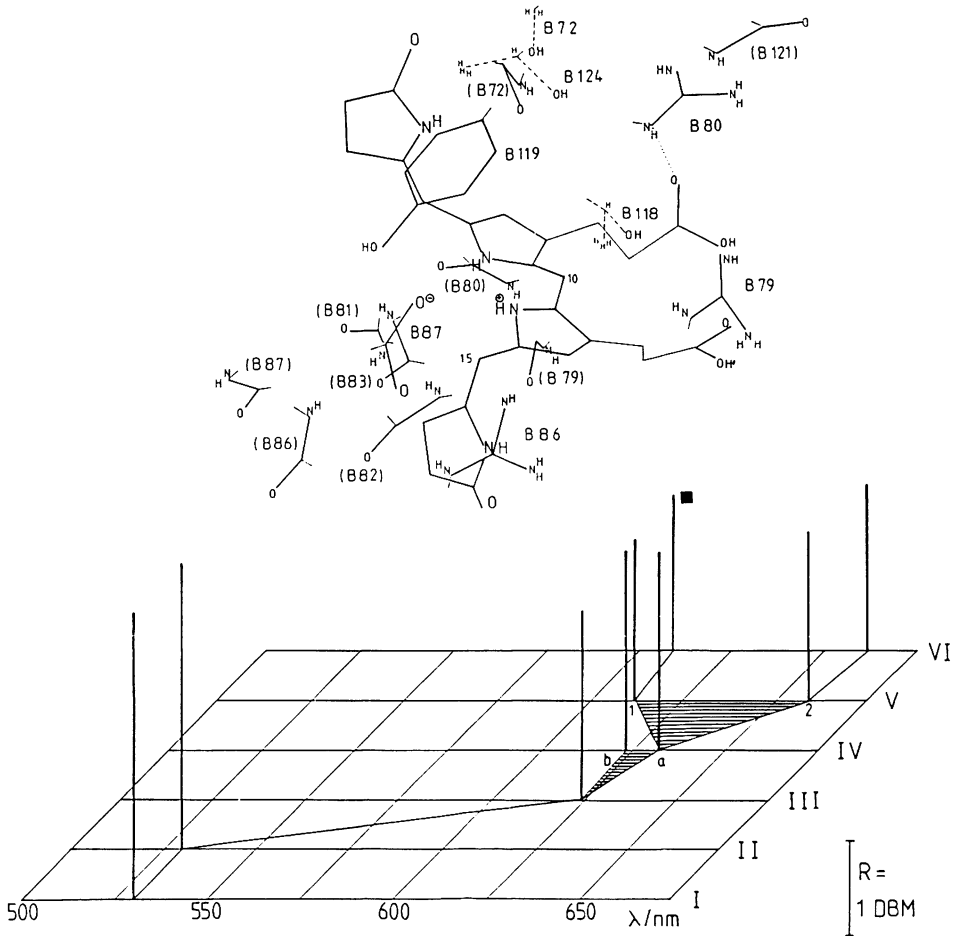
Case V: IV + all polar and aromatic residues in a sphere of  
10 Å around C10

Case VI: V + amide groups of the protein backbone within the  
same sphere (in brackets)

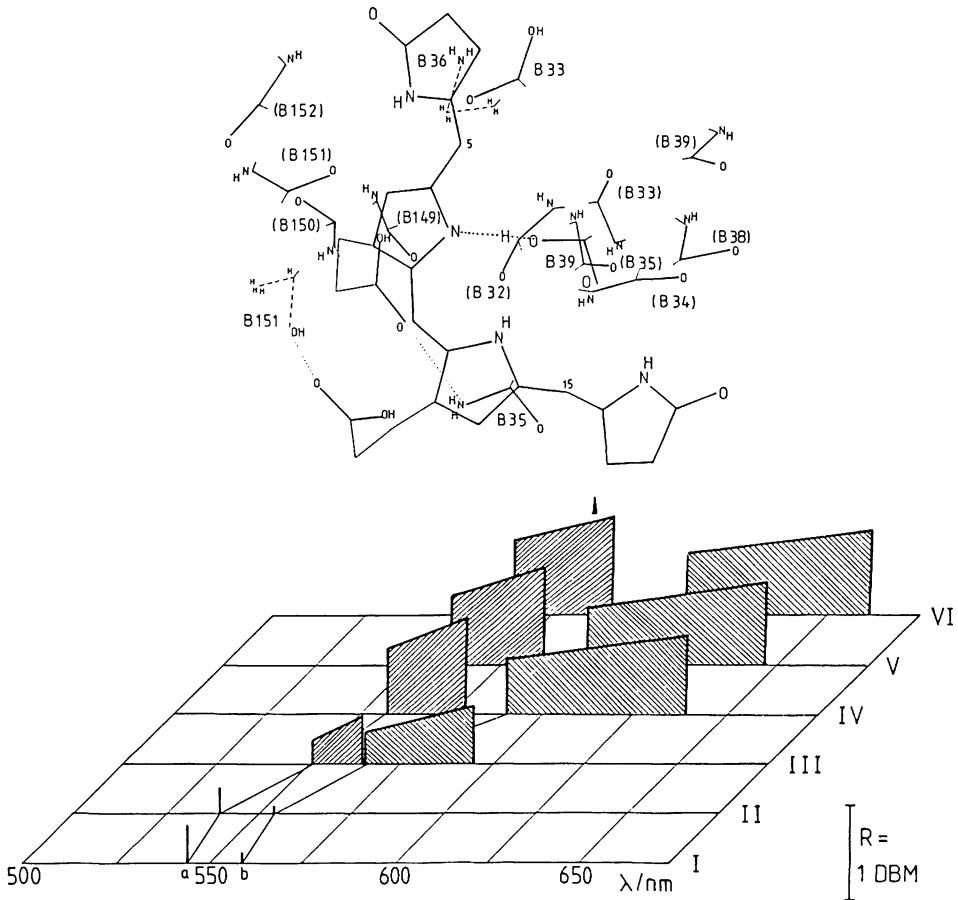
The calculated transition energies for the isolated chromophores (I) are around or below 550 nm. The origin for this hypsochromic shift, when compared to prior calculations (6a,6b,7), is the significant deviation from a planar geometry. Coulombic interaction (static coupling in terms of first order perturbation theory) effects the transition energies (bathochromic shift) mainly because of the different stabilization of the various molecular orbitals. The rotatory strength is altered because of changed coefficients in the CI-wavefunctions and the concomitant modified mixing of electric and magnetic transition moments.

Inclusion of the carboxylate groups of the propionic acid side chains (II) exhibits only small effects (the proton tautomerism is fixed by the interaction with adjacent amino acids (3c)).

Both chromophores (as well as the third, not displayed, at A84) show a common principle of interaction with the protein.



**Figure 1:** Calculated rotatory strength  $R$  of the longwavelength absorption band of the **B84** chromophore ( $1 \text{ DBM} = 0.93 \cdot 10^{-23} \text{ cgs}$ ; case I to VI; see text). The shaded regions show the spectral variations with the tautomerism of the arginine groups:  
 a) B80 as shown                      b) tautomeric form  
 1) B79 and B86 as shown        2) tautomeric form



**Figure 2:** Calculated rotatory strength  $R$  of the longwavelength absorption band of the B155 chromophore. The shaded area reflects the bathochromic shift calculated for an increasing of the hydrogen bond strength to the B39 aspartate residue.  
 a) N23-H-tautomer                      b) N22-H-tautomer

They arch around an aspartate residue (B87 and B39, resp.), the nitrogens of pyrrole rings B and C (N22 and N23) being within hydrogen bonding distance of one of the carboxylate oxygens. This interaction dominates the wavelength adjustment (III).

For B84 (and A84) the assumption of a proton transfer to the chromophore, leaving a negative aspartate counterion, is necessary to shift the calculated electronic excitation energy into the experimentally established region.

B155 deviates from this behaviour; a proton transfer would result in a transition near 700 nm. Therefore we postulate that B155 and aspartate B39 are coupled via a hydrogen bond. Only a positive partial charge near N22 and N23 (OD2 treated as -O-H) yields a redshift. Depending on the methen tautomerism (N22-H or N23-H) and the hydrogen bond strength, the visual absorption band varies in a wavelength region of about 50 nm width (shaded areas in figure 2).

Inclusion of the charged and aromatic residues around B84 (case IV: Arg B80; case V: Arg B79, Arg B86, Ser B72, Tyr B119, Thr B124, Thr B118) leads to further shifts. There is a strong indication, that the arginine residues play a vital role in tuning the transition energy. Small variations in geometry (e.g. a rotation of the  $-C(NH_2)(NH)-$ groups by  $180^\circ$ , equivalent to an exchange of positive and negative partial charges) result in a drastic variation of the transition wavelength. Therefore, these residues may be responsible for the heterogeneity observed in the fluorescence kinetics of smaller aggregates (8).

Inclusion of the charges in the surrounding of B155 (case IV: Thr B151, Asn B35; case V: Lys B36, Glu B33) induces also additional redshifts of the first electronic transition. Only for the N23-H-tautomer is the theoretical result, however, in agreement with observation.

The interaction with the amide groups of the protein backbone (case VI) produces only small corrections to the excited state properties and can therefore be ignored in first approximation studies.

$\beta$ -subunit

The results described so far predict that the CD-spectrum of two, non-interacting chromophores in the  $\beta$ -subunit should exhibit a large rotatory strength around 620 nm. This is, however, in contradiction to the experimental finding (4) (inactive longwavelength absorption band, attributed to B84). Therefore, we extended our model calculations to take into account both chromophores and their environments together. The selected status of chromophore-protein interaction according to approximation VI is marked in figures 1 and 2 for both chromophores. It yields transition energies in the correct wavelength region and also the required energy spacing for B84 and B155. Figure 3 shows the thereby obtained theoretical CD-spectrum of the  $\beta$ -subunit.

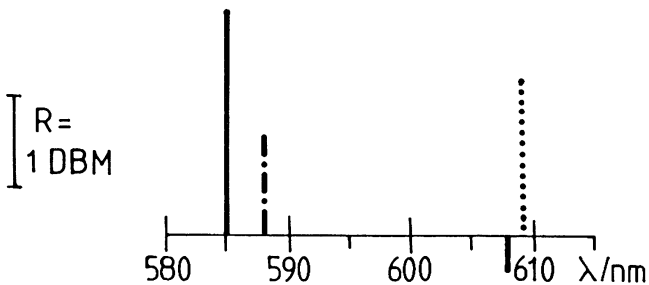


Figure 3: Calculated visible CD-spectrum of individual chromophores (B84 ..... , B155 ----) and the  $\beta$ -subunit with excitonic coupling included (——).

Due to excitonic coupling, the shorter wavelength transition ( $\psi^+ = 0.9 \psi^*(B155) + 0.1 \psi^*(B84)$ ) gains rotatory strength, which is lost in the corresponding longwavelength transition. This final result fits the experimentally found CD-bandshape very well and is a clear indication, that the interaction with charged residues controls also the chromophore-chromo-

phore coupling within the phycobiliproteins. It is furthermore a warning that the properties of isolated chromophores may not be extracted from measurements on biological systems with interacting chromophores.

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