Table 1. Spectral and temporal parameters from six strains of cyanobacteria.

Strain	Parameters							
	A_{495}/A_{545}* for isolated phycobilisomes	α_1	τ_1 (psec)	f_1†	α_2	τ_2 (psec)	f_2†	$\frac{\alpha_2}{\alpha_2 + \alpha_1}$
WH8103	1.83	1.0	2230	0.59	8.7	180	0.41	0.90
WH8112	0.79	1.0	2010	0.39	23	136	0.61	0.96
WH8020	0.65	1.0	1790	0.17	84	107	0.83	0.99
WH7803	0.36	1.0	2010	0.34	41	94	0.66	0.98
WH7805	0.17	1.0	2000	0.34	22	180	0.66	0.96
PCC6701	0.14	1.0	2220	0.55	37	50	0.45	0.97

*The ratio A_{495}/A_{545} is related to the bilin chromophore content of the PE complexes of the strain. For instance, for strain WH8103 there are 21 phycourobilin chromophores (λ_{max}, 490 nm) and 13 phycoerythrobilin chromophores (λ_{max}, 545 nm) per $(\alpha\beta)_6\gamma$. In contrast, the PE of strain WH7805 and of the freshwater strain, PCC6701, contains no phycourobilin chromophores. †The f_i give the fractional yield of integrated emission for the i^{th} component.

free PE is about 2 nsec, PE within phycobilisomes is quickly quenched without radiation by energy transfer and shows a fluorescence lifetime of about 0.1 nsec. Because of the lifetime bias, the steady-state signal detects contributions from the free PE 20 times more sensitively than does the signal from bound PE. Small changes in the degree of association toward lesser association can cause large decreases in apparent "coupling efficiency" when measured as in (*4*). For instance, with a bound PE lifetime of 0.1 nsec and a free PE lifetime of 2 nsec, a change in fraction of bound PE as small as 3% (from 1.0 to 0.97) can give a decrease of 40% in the apparent "coupling efficiency" (*9*). The same change in apparent "coupling efficiency" would result from a change in the fraction of bound PE from 0.43 to 0.01. Although the change in measured "coupling efficiency" is the same for both of these cases, the change in the fraction of absorbed photons arriving at the reaction center is radically different (3% decrease for the first case, and a 98% decrease for the second). These considerations lead us to conclude that the results of Wyman *et al.* (*4*) are not necessarily in conflict with those we report here and may be the consequence of small changes in the intracellular levels of free PE.

SHEILA WALSH YEH
Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory AND *Department of Chemistry, University of California, Berkeley, CA 94720*
LINDA J. ONG
ALEXANDER N. GLAZER
Department of Microbiology and Immunology, University of California, Berkeley, CA 94720

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9. Following the paper by Wyman *et al.*, a "coupling efficiency" is defined as

$$\eta = \frac{F_{570,g}}{F_{570,ng}}$$

where η is the coupling efficiency and F_{570} is emission intensity at 570 nm. The signals are measured from cultures that have either been treated by the addition of glycerol, or without glycerol added. Because glycerol uncouples phycoerythrin from energy transfer, $F_{570,g}$ is a measure proportional to the total amount of of phycoerythrin in the sample $F_{570,g} = k\ PE_t$, where PE_t is total phycoerythrin. Complicating the interpretation of η, the signal $F_{570,ng}$ has components from both free phycoerythrin, PE_f, and phycoerythrin bound in phycobilisomes, PE_b. The two phycoerythrin components contribute to the signal in proportion to their concentration and their fluorescence lifetime. Therefore

$$F_{570,ng} = \tau_f PE_f + \tau_b PE_b$$

where τ is the emission lifetime. Defining the ratios PE_f/PE_t and PE_b/PE_t as α_f and α_b, the fractions of free and bound phycoerythrin in the cultures, the previously defined "coupling efficiency" can be written

$$\eta = \frac{k}{\tau_f(1 - \alpha_b) + \tau_b\alpha_b}$$

Wyman *et al.* related directly the change measured in the "coupling efficiency" to changes in the fraction of bound phycoerythrin between cultures grown with sufficient nitrogen (η^H, high nitrogen) and those that were nitrogen-starved (η^L, low nitrogen). However, the relation between the fraction bound and the reported "coupling efficiency" is not uniquely defined. The following equation results from simplifying algebraically the ratio η^H/η^L:

$$\alpha_b^L = \left(\frac{\eta_H}{\eta^L}\right)\alpha_b^H + \left(1 - \frac{\eta^H}{\eta^L}\right)\left(\frac{\tau_f}{\tau_f - \tau_b}\right)$$

For the measurement in the report of Wyman *et al.*, $\eta^H/\eta^L = 0.6$, so

$$\alpha_b^L = 0.6\alpha_b^H + 0.4\left(\frac{\tau_f}{\tau_f - \tau_b}\right)$$

10. We thank J. B. Waterbury for providing axenic cultures of the marine *Synechococcus* spp. S.W.Y. acknowledges support from Amoco Corporation. L.J.O. was supported by a fellowship from the Department of Health and Human Services Training Grant 5T32 GM7232-09. Supported by National Science Foundation grant DMB-8518066.

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Response: Yeh *et al.* (*1*) raise questions concerning the validity of our estimations (*2*) of the relative light-harvesting (coupling) efficiency of phycoerythrin in cells of *Synechococcus* strain DC2. On the basis of the assumption that energy transfer is 100% efficient in nitrogen-limited cells, Yeh *et al.* suggest that only a small fraction (3%) of phycoerythrin is uncoupled from energy transfer in high-nitrogen grown cells. However, the accepted viewpoint in the literature (*3*) suggests that energy transfer from biliproteins to the reaction center of photosystem II is not 100% efficient. If it is assumed, for instance, that the maximum possible efficiency of energy transfer is 90% to 95% (*3*), then solving the final equation in reference 9 of Yeh *et al.* leads to a value of 13% to 22% for the amount of uncoupled phycoerythrin present in high-nitrogen grown cells under our growth conditions. We view this as a significant figure. Yeh *et al.* have used a culture medium with a nitrogen concentration similar to our own, but they have produced their experimental material under radically different growth conditions (batch as opposed to continuous culture). While insufficient information is given for us to draw firm conclusions, a possible reason why they did not observe greater than 10% "free" phycoerythrin in their cell material may be that they used cultures which were light-limited at harvest. In our original report (*2*) we stressed that it was the interaction between the availability of light and nitrogen which determined the amount of uncoupled phycoerythrin present, and we demonstrated, as Yeh *et al.* have found, that only a relatively small percentage of phycoerythrin is uncoupled in high-nitrogen cells grown under low light intensities.

While Yeh *et al.* have specifically addressed the question regarding the light-harvesting efficiency of phycoerythrin, there remain other lines of evidence which we presented in support of our hypothesis that this biliprotein serves as a reserve of stored nitrogen as well as a component of the light-harvesting apparatus. We showed that the rate of photosystem II activity was similar in high- and low-nitrogen grown cells, although the cell concentrations of phycoerythrin were significantly different under these

two growth regimes. Furthermore, when the availability of external nitrogen is interrupted, phycoerythrin is mobilized in high-nitrogen grown cells and growth rate is maintained. Since the difference in the phycoerythrin content of nitrogen-replete and nitrogen-limited cells is considerable, it was not part of our original argument that only energetically uncoupled phycoerythrin is mobilized during nitrogen starvation. Rather, we see the accumulation of uncoupled phycoerythrin as one part of a reserve which continues to meet the nitrogen demands of the cell during short-term periods of reduced nitrogen availability (*4*).

M. WYMAN
Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, Great Britain
R. P. F. GREGORY
Department of Biochemistry, University of Manchester, Manchester M13 9PL, Great Britain
N. G. CARR
Department of Biological Sciences, University of Warwick

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Magnetically Oriented Solutions

The report "X-ray diffraction from magnetically oriented solutions of macromolecular assemblies" by Glucksman *et al.* (*1*) does not aknowledge that the virus solutions investigated are liquid crystal phases (*2–4*). The field-induced orientation reported would not be possible were it not for the preexisting high degree of orientational ordering of the virus particles indigenous to such phases.

The liquid crystallinity of the Pf1 solutions examined by Glucksman *et al.* is evident from figure 2 of their report; the virus solution external to the field is birefringent. This spontaneous birefringence is a direct consequence of long-range orientational ordering of the particles that extends over macroscopic distances in liquid crystals. It is usually expressed with an order parameter, S, an average over the restricted, rotational diffusion of the rods within a uniform (macroscopic) domain. In typical liquid crystals, values of S are in the range of 0.3 to 0.9. This high intrinsic order accounts for the spontaneous birefringence of liquid crystals even in the absence of external constraints (shear, surface effects, applied magnetic or electric fields, and so forth), where the local nematic director (optic axis of a domain) assumes random orientations throughout a bulk sample.

It has been recognized for a long time that random liquid crystal textures may be readily transformed into uniform textures [a "single (liquid) crystal"] by exposing them to modest magnetic fields [$H \leq 0.2$ tesla (*5*)]; the feasibility of magnetic alignment in macromolecular liquid crystals despite their higher viscosities was reported two decades ago (*6*). The phenomenon derives from the interaction of the field with the bulk diamagnetic anisotropy of the ordered fluid, $\Delta\chi$ (*7*), and not with the anisotropy of an individual particle, $\Delta\chi°$, as implied. The magnetic potential energy for typical macromolecular assemblies, $\Delta\chi°H^2$, is small relative to ambient thermal energy. Even high-strength fields ($H \cong 10$ T) induce small orientational biasing of the Brownian motion of isolated particles suspended in isotropic, dilute solutions, that is, the classical Cotton-Moutton effect, where, dependent on the magnitude of $\Delta\chi°$, S is observed to be 10^{-6} to 10^{-5} for organic molecules (*8*), and is typically $< 10^{-2}$ for macromolecular assemblies (*4*).

While the magnet design proposed by Glucksman *et al.* ($H < 2$ T) can facilitate diffraction experiments, the large degree of orientational order required to carry out high-resolution structural analysis ($S > 0.5$) will limit its use to studies of liquid crystal phases. Generally speaking, for biological macromolecules or macromolecular assemblies, the prerequisites for liquid crystallinity will in turn limit consideration to solutions (with $\phi > \phi^*$) of those species having anisometric shapes. Particles with large aspect ratios (prolate or oblate) are required for lyotropic liquid crystal formation.

E. T. SAMULSKI*
Cavendish Laboratory, Cambridge University, Madingley Road, Cambridge CB3 OHE, United Kingdom

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*Present address: LCP Research Center, U-136 IMS, University of Connecticut, Storrs, CT 06268.

Response: We agree with most of the points raised by Samulski and appreciate his detailed discussion of the importance of long-range orientational ordering as a prerequisite for obtaining highly oriented phases. As we discuss in the last paragraph of our report, particles with anisotropic shapes may orient better because the orientations of adjacent particles are not independent of one another. In fact this may be required for formation of highly oriented specimens. Exposing a liquid crystalline texture to a modest magnetic field does not universally result in the formation of highly oriented specimens, as Samulski suggests. This depends on the properties of the macromolecular assembly of interest. The orientation of a liquid crystalline domain depends on its bulk diamagnetic anisotropy, but the bulk anisotropy depends, in turn, on the anisotropy of individual particles and the orientational correlation among particles in the domain. Our interest here is in the properties of the individual particles and the generation of high-quality diffraction data. Samulski's interest appears to be in the bulk behavior of liquid crystalline textures. His point of view supplements our work, and his comments are appreciated.

LEE MAKOWSKI
MARC GLUCKSMAN
Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032

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