with which parallel assays may be carried out is also significant. This assay is appropriate as described for activity screening of hybridomas in place of ELISA screening for binding. Minor modifications would allow use in other formats.

The same convenient procedure, with very similar reagents, may be used to follow diverse classes of concatenation reactions. For example, while we have demonstrated the detection of peptide coupling, there is no apparent reason that a Diels-Alder or other reaction would not also be amenable to this approach.

Recent advances in combinatorial antibody technology[11] and other strategies[12] promise to provide an even greater repertoire of potential catalysts, placing greater demands on the screening procedures. We have shown that a highly sensitive and generally applicable assay is now available for the detection of low concentrations of catalysts for bimolecular reactions.

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(13) Abbreviations: ABTS, diammonium 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate); HRP, horseradish peroxidase; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; PBS, 10 mM pH 7.2 phosphate, 150 mM NaCl; SDS, sodium dodecyl sulfate.

Mimicking Carotenoid Quenching of Chlorophyll Fluorescence

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Carotenoid pigments are ubiquitous in photosynthetic membranes. They act as antennae by transferring singlet energy into the chlorophyll manifold to drive photosynthesis and as photoprotective pigments by transferring triplet energy out of the chlorophyll system, thereby preventing chlorophyll-sensitized singlet oxygen formation.[1-3] Carotenoids also quench chlorophyll fluorescence in vitro[4] and in vivo.[5] This quenching presents a paradox: carotenoids inject excitation energy into the chlorophyll singlet manifold, but they then dissipate it before it can be used for photosynthetic work. Synthetic carotenoporphyrins mimic carotenoid energy transfer and exhibit carotenoid quenching of tetrapyrrole fluorescence.[3,6] We now report that in a specially designed carotenoporphyrin, quenching results from electron transfer from the carotenoid to the excited singlet porphyrin followed by fast charge recombination.

Although work reporting the singlet lifetimes and fluorescence and energy-transfer properties of carotenoids has recently appeared, the mechanisms whereby these pigments quench the excited singlet states of cyclic tetrapyrroles remain obscure.[7-16]

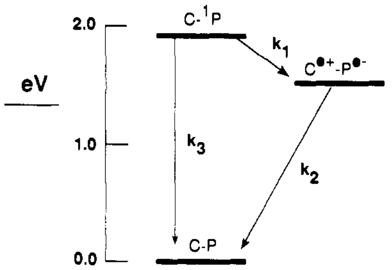


Figure 1. Energy levels and relaxation pathways for the porphyrin first excited singlet state of **1**. Charge separation k_1 and recombination k_2 are electron-transfer processes; k_3 refers to the sum of the other relaxation processes from the first singlet excited state (measured in **2**).

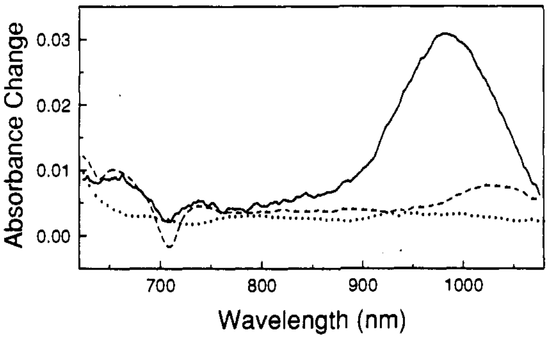
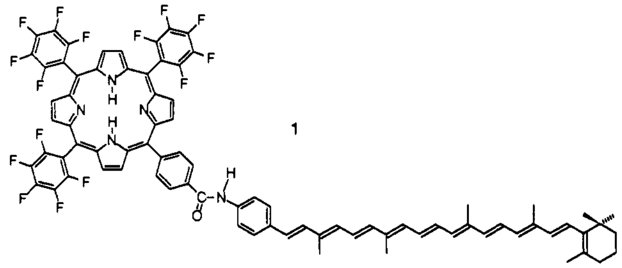


Figure 2. Transient absorption spectra in butyronitrile of **1** (—) and **2** (---) taken 10 ps after a ca. 150-fs, 5-mJ laser flash (108K flashes) and **3** (···) taken 3 ps after the flash. The absorbance of **1** was 2 per cm (ca. 3.6×10^{-4} M) at the excitation wavelength of 590 nm, where 90% of the light is absorbed by the porphyrin. The absorbance of **2** was 1.8 per cm, and that of **3** was 0.2 per cm, so the populations of excited porphyrin and carotenoid in **2** and **3** are the same as those produced in **1**.

Thermodynamically, quenching could occur by electron transfer from the carotenoid (C) to the cyclic tetrapyrrole (P) first excited singlet state to yield a charge-separated (CS) species.[4] Singlet energy transfer from the cyclic tetrapyrrole to the electric-dipole-forbidden S_1 state of the carotenoid is also a possibility, although the energetics of that process are unknown.[13-16]



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Carotenoporphyrin dyad **1** was designed to maximize the possibility of observing the CS species from electron-transfer

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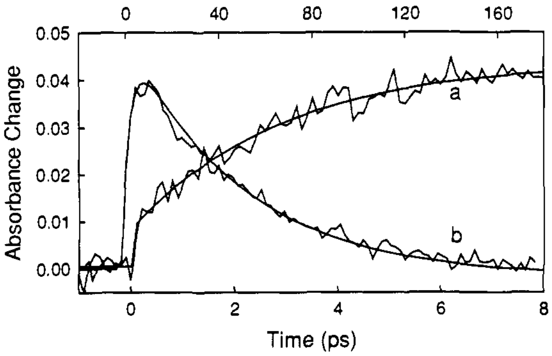

Figure 3. (a) Rise of the carotenoid radical cation species in **1** after excitation measured as an absorbance change centered at 960 nm (0.1-ps increments, lower axis). Regression analysis yields a time constant of 2.9 ps. (b) Rise and decay of the carotenoid radical cation signal (2-ps increments, upper axis). The decay time constant is 51 ps.

quenching. The electronics interaction required for electron transfer is provided by the amide linkage.[6] The necessary driving force is furnished by the high electron affinity of the fluorinated porphyrin. Cyclic voltammetric measurements locate the CS-state energy ca. 420 meV below that of the porphyrin excited singlet state (Figure 1). The absorption spectrum of **1** is essentially a linear combination of those of models **2** (5-(4-carbomethoxyphenyl)-10,15,20-tris(pentafluorophenyl)porphyrin) and **3** (7-apo-7′-(4-benzamidophenyl)-β-carotene), indicating that interchromophore interaction is weak. ^{1}H-NMR conformational studies[17] show that the carotenoid extends out, away from the porphyrin.

The fluorescence of **1** derives from the porphyrin component and is indistinguishable from that of **2** except in quantum yield. The fluorescence excitation spectrum demonstrates that excitation of the carotenoid in **1** results in 17% efficient singlet energy transfer to the porphyrin. From fluorescence decay measurements of **1** and **2**, the rate constants k_1 for quenching of the porphyrin first excited singlet state were found to be 1.3×10^7, 2.1×10^9, and 1.8×10^{10} s^{-1} in *n*-hexane, toluene, and butyronitrile, respectively (the fluorescence lifetimes of **2** were 8.3, 9.7, and 11.0 ns in these solvents). Because CS-state energy decreases with solvent dielectric constant and theory[18] predicts a corresponding electron-transfer rate increase,[19] these results are indicative of electron transfer. In low dielectric solvents the porphyrin triplet yield is significant and rapid triplet energy transfer to populate the carotenoid triplet is observed.

Transient absorption spectra of **1**, **2**, and **3** in butyronitrile taken after laser excitation are presented in Figure 2. All three compounds show the expected excited-singlet-state features, including prominent porphyrin stimulated emission at ca. 710 nm for **1** and **2**. The spectrum of dyad **1** is dominated by a transient absorption at 975 nm. This band rises with a time constant of 2.9 ps (Figure 3a), decays with a lifetime of 51 ps (Figure 3b), and is characteristic of the carotenoid radical cation ($\lambda_{max} \sim 960$ nm in dichloromethane, $\sim$1025 nm in benzene, $\epsilon \sim 2 \times 10^5$ M^{-1} cm^{-1}).[20]

The direct observation of $C^{\bullet+}$–$P^{\bullet-}$ by the characteristic absorption of the carotenoid radical cation confirms electron-transfer quenching in **1**. The kinetic behavior of the $C^{\bullet+}$–$P^{\bullet-}$ transient appears unusual in that its decay time of 51 ps correlates with the 54-ps lifetime of the porphyrin singlet precursor while its rise time is much faster (ca. 3 ps). However, this behavior is expected if $k_3 \ll k_1$ and $k_2 \gg k_1$ (Figure 1). Moreover, there is a special signature for $k_2 > k_1$. Although the yield of $C^{\bullet+}$–$P^{\bullet-}$ is essentially 1 (quenching is >99%), the maximum concentration of the CS species is reduced from that of the initial C–^{1}P by k_1/k_2. We calculate from ϵ of $C^{\bullet+}$–$P^{\bullet-}$ and the fraction of C–P excited to C–^{1}P that the observed absorbance change is indeed reduced by ca. 20-fold from that expected for $k_1 \gg k_2$. Therefore, the quenching of C–^{1}P is assigned to electron transfer yielding $C^{\bullet+}$–$P^{\bullet-}$ with a formation time of just over 50 ps ($k_1 \sim 1.8 \times 10^{10}$ s^{-1}) and a lifetime of ca. 3 ps ($k_2 \sim 3.3 \times 10^{11}$ s^{-1}).

The transient species characteristic of energy-transfer quenching in **1** were not observed. Moreover, since energy transfer in these systems is not expected to be strongly solvent dependent, the negligible quenching observed in *n*-hexane sets an upper limit for the energy-transfer process. In other types of carotenoid interactions with cyclic tetrapyrroles energy transfer or other quenching mechanisms could occur.[13-16]

In conclusion, the observation of porphyrin fluorescence quenching by electron transfer from a carotenoid provides a mechanism for similar energy-dissipating electron-transfer quenching in vivo[5] and warns that because of the kinetics it could be very difficult to detect the transient species in pigment-bearing proteins. A rapid charge recombination process would be advantageous because it would preclude deleterious reactions of the radical ions in the photosynthetic membranes. Through energy dissipation, carotenoids could play a role in controlling photosynthetic energy flow.

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Selective Reduction of Steroid 3- and 17-Ketones Using LiAlH$_4$ Activated Template Polymers

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Extensive work, particularly by Wulff and his associates, has shown that molecular cavities with regularly spaced functional groups may be created with the molecular imprinting technique.[1] It has also been shown by Mosbach and his co-workers that shape selective cavities may be prepared by a similar technique.[2] Both techniques have been used for chromatographic separation of enantiomers and other types of molecular recognition.[3] For some

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