between the metal ions is small as shown by the unresolved, single $Ru^{III/II}$ wave at $E_{1/2}$ = 1.16 V.[12]

The lifetime of the bbpe-based MLCT state in $[(dmb)_2Ru(\mu\text{-bbpe})Ru(dmb)_2]^{4+}$, 1.31 ± 0.05 μs in CH_3CN at 298 ± 2 K, is surprisingly long. Lifetimes of MLCT excited states characteristically decrease with the energy gap between the excited and ground states.[2] Yet, for $[Ru(dmb)_3]^{2+}$, which has a significantly higher energy gap, E_{em} = 15 600 cm^{-1} compared to 13 300 cm^{-1}, the lifetime is 950 ± 30 ns under the same conditions. For $[Ru(bpy)_2(py)Cl]^+$ (py = pyridine), where there is a comparable emission energy (752 ± 5 nm, 13 300 cm^{-1}), the lifetime is 44 ± 3 ns under the same conditions. On the basis of the results of temperature-dependent lifetime studies, at 157 ± 2 K in 4:1 (v:v) ethanol-methanol, there are no complications from low-lying dd states.[16] Under these conditions, lifetimes, as determined by emission decay, were 1.97 ± 0.07 μs for $[(dmb)_2Ru(\mu\text{-bbpe})Ru(dmb)_2]^{4+*}$ and 104 ± 4 ns for $[Ru(bpy)_2(py)Cl]^{+*}$.

The extended lifetime for the bbpe complex appears to be a consequence of electronic delocalization in bbpe acting as the acceptor ligand. Emission spectral profiles in 4:1 (v:v) ethanol-methanol at 157 ± 2 K or 298 ± 2 K could be fit[13] satisfactorily by including contributions from an averaged ν(bpy) mode ($h\nu$ = 1350 cm^{-1}), an averaged low-frequency mode ($h\nu$ = 350 cm^{-1}), and the solvent. On the basis of this analysis, the electron-vibrational coupling constant, S, for ν(bpy) was 0.72 ± 0.05 for $[Ru(bpy)_2(py)Cl]^{+*}$ and 0.60 ± 0.05 for $[(dmb)_2Ru(\mu\text{-bbpe})Ru(dmb)_2]^{4+*}$. The quantity S is related to the change in equilibrium displacement between the excited and ground states, ΔQ_e, the reduced mass, M, and the angular frequency, ω ($=2\pi\nu$), by eq 2. On the basis of the smaller value of S, the structural

$$S = (1/2)(M\omega/h)(\Delta Q_e)^2 \quad (2)$$

change in the acceptor ligand is decreased for $(dmb)_2Ru(\mu\text{-bbpe})Ru(dmb)_2]^{4+*}$. This is a further indication that delocalization over both bpy's has occurred. The decrease in S and ΔQ_e with enhanced delocalization must be a consequence of decreased electron-electron repulsion in the acceptor ligand.

The decrease in S is sufficient to account, to a large degree, for the enhanced lifetime in $[(dmb)_2Ru(\mu\text{-bbpe})Ru(dmb)_2]^{4+*}$ compared to $[Ru(bpy)_2(py)Cl]^{+*}$ at 157 K. From radiationless decay theory and the energy gap law,[14] the nonradiative decay rate constant, k_{nr}, is predicted to vary with S, the energy gap (E_0), and $h\nu$ as shown in eq 3.[13b,14] For $h\nu$ = 1350 cm^{-1}, E_0 = 13 780

$$k_{nr} \propto e^{-S}e^{-\gamma E_0/h\nu} \quad (3)$$

$$\gamma = (\ln E_0/Sh\nu) - 1$$

cm^{-1}, and S = 0.72 or 0.60, the decrease in k_{nr} between $[Ru(bpy)_2(py)Cl]^{+*}$ and $[(dmb)_2Ru(\mu\text{-bbpe})Ru(dmb)_2]^{4+*}$, calculated by using eq 3, is 17 in 4:1 (v:v) ethanol-methanol at 157 ± 2 K. The parameters used in the calculations were those obtained by spectral fitting. The decrease observed experimentally was 19. The origin of the effect is a decrease in vibrational overlap between the vibrational wave functions for the ν(bpy) acceptor modes between the excited and ground states as ΔQ_e decreases.

Compared to related complexes having comparable energy gaps, we find that lifetimes for the MLCT excited states of $[Ru(dmb)_2(bbpe)]^{2+}$, $[Os(bpy)_2(bbpe)]^{2+}$, $[(bpy)_2Os(\mu\text{-bbpe})Os(bpy)_2]^{4+}$, $[Re(bbpe)(CO)_3Cl]^+$, or $[Cl(CO)_3Re(\mu\text{-bbpe})Re(CO)_3Cl]^{2+}$ are all lengthened. These observations and the sometimes anomalous lifetime behavior observed for other MLCT excited states[10,15] point to what could be a general approach to enhancing excited-state lifetimes based on introducing increased delocalization in the acceptor ligand.

Our results have important implications for the design of MLCT based photosensitizers. They open the possibility of preparing complexes that have significant light absorptivities much further into the red while, at the same time, maintaining lifetimes that are accessible to electron-transfer quenchers.

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Supplementary Material Available: The preparations and characterizations of the bbpe ligand and complex (3 pages). Ordering information is given on any current masthead page.

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Putidaredoxin Reduction of Cytochrome $P\text{-}450_{cam}$: Dependence of Electron Transfer on the Identity of Putidaredoxin's C-Terminal Amino Acid

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Considerable experimental and theoretical efforts have been made to determine the effect of exothermicity, distance, and protein medium on the rate of biological electron transfer.[1,2] We have discovered that the interprotein electron transfer from site-specific variants of putidaredoxin (a 2Fe-2S ferredoxin) is strongly affected by the presence or absence of a C-terminal aromatic residue. Activity is maintained when the wild-type C-terminal tryptophan is replaced by other aromatic residues but is lost upon nonaromatic replacement or deletion.

Putidaredoxin functions in the *Pseudomonas putida* camphor hydroxylase electron-transfer chain as a one-electron shuttle between an NADH-dependent flavoprotein and the terminal acceptor, cytochrome $P\text{-}450_{cam}$. This enzyme system catalyzes the first catalytic step whereby *P. putida* ATCC strain 17453 can use camphor as its sole source of carbon and energy.[3] The C-terminal tryptophan of putidaredoxin is known to be solvated, independently mobile, accessible to carboxypeptidase cleavage, and near an anionic surface domain.[4] Preliminary NMR structure

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Table I[a]

putidaredoxin variant	redn potential, mV	dissocn const, μM	rel rate of NADH oxidn
WT 106 Trp	−233	5.4	1.00
106 Phe	−236	5.2	0.44
106 Tyr	−239	5.2	0.20
106 Asp	−259	5.8	<0.02
106 Leu	−235	5.2	<0.02
106 Lys	−223	4.7	<0.02
106 Val	−238	5.4	<0.02
106 deletion	−241	10.9	<0.02

[a] The reduction potentials were determined by measuring the concentrations of oxidized putidaredoxin and phenosafranine (E_0 = −253 mV) at equilibrium after partial photoreduction.[14] The concentrations of putidaredoxin and dye were each 30 μM in 10 mM EDTA and 50 mM Tris·HCl pH = 7.35. The error in each measurement is less than 5 mV. The dissociation constants were determined by titrating putidaredoxin with fluorescent probe labeled P-450$_{cam}$.[15] Titrations of each variant putidaredoxin involved 10 successive additions of 0.50 nmol of putidaredoxin to 0.50 nmol of labeled P-450$_{cam}$ in 1.0 mL of 10 mM MOPS, pH = 7.15 at 20 °C, with standard analysis.[16] Rates of NADH oxidation were measured spectrophotometrically at 340 nm in the presence of 7.1 μM putidaredoxin, 0.35 μM putidaredoxin reductase, 0.04 μM P-450$_{cam}$, 143 mM KCl, and 107 μM NADH and in the absence or presence of 159 μM camphor. The relative rates are expressed as the initial velocity for the variant in the presence of camphor minus the initial velocity without camphor divided by the same for WT 106 Trp.

determination (Pochapsky et al., unpublished) suggests that the C-terminus lies above the carboxylate domain implicated in complex formation with its redox transfer partners.[5] We manipulated the gene encoding putidaredoxin to produce variants where the C-terminal tryptophan is replaced by Asp, Leu, Lys, Phe, Tyr, or Val or is deleted.[6] These mutations were introduced without markedly affecting the reduction potential or redox partner binding energy (Table I).

The activities of the variant putidaredoxins were determined in two separate assays. First, we examined the rate of NADH oxidation in the reconstituted camphor hydroxylase electron-transfer chain, Table I. In the absence of camphor, the NADH oxidation rate represents background autoxidation activity independent of P-450$_{cam}$.[7] For the nonaromatic mutants, no measurable camphor-dependent oxidation of NADH was detected. A second and more specific measure of putidaredoxin's activity is its rate of interprotein electron transfer with complexed ferric P-450$_{cam}$ under anaerobic conditions.[8,9] A performed complex of putidaredoxin and P-450$_{cam}$ was reduced by flash photolysis at 355 nm in an anaerobic solution containing 10 μM putidaredoxin, 10–55 μM P-450$_{cam}$, 25 μM acridine, 500 μM methylviologen, 25 mM EDTA, 50 mM Tris·HCl pH = 7.5.[8,10] Under these conditions, the resulting photogenerated methylviologen radicals (1–2 μM) reduce putidaredoxin (10 μM) some 200 times faster than P-450$_{cam}$. Equilibration of the reduced putidaredoxin–P-450$_{cam}$ complex is fast relative to the P-450$_{cam}$ reduction step.[9] The change in reduced P-450$_{cam}$ concentration as a function of time fits a single-exponential decay yielding the observed rate constants given in Figure 1.

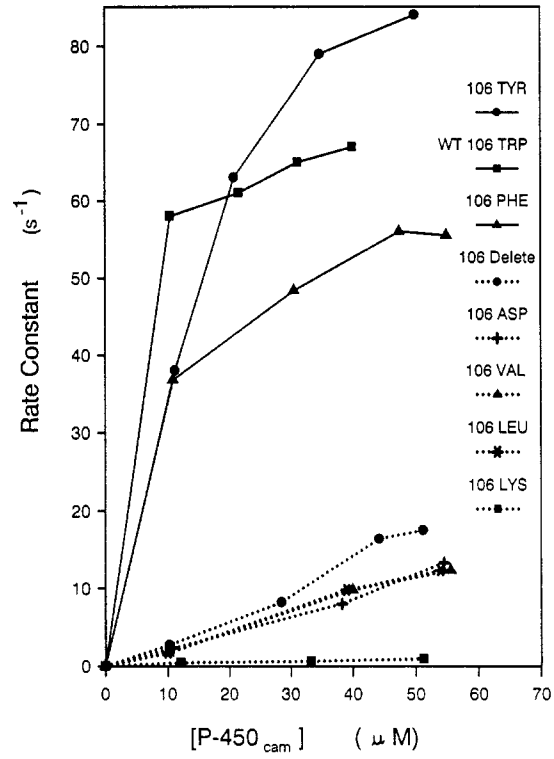

Figure 1. Plot of first-order rate constants for reduction of P-450$_{cam}$ by putidaredoxin variants as a function of P-450$_{cam}$ concentration. Rates for wild-type and variants with C-terminal aromatic residues are shown with solid lines; variants with nonaromatic residues are shown with dotted lines.

As seen from Figure 1, the rate constants measured for the nonaromatic variant putidaredoxins are greatly reduced but do not saturate at high concentrations of P-450$_{cam}$. This implies that a second-order process occurs that is independent of putidaredoxin binding. If the reduction of P-450$_{cam}$ by putidaredoxin was slow relative to the rate of methylviologen reduction of P-450$_{cam}$, putidaredoxin would be expected to equilibrate with P-450$_{cam}$ either at the rate of methylviologen radical reduction of P-450$_{cam}$ or at the rate of putidaredoxin reduction of methylviologen, whichever is slower. Consistent with this, the apparent second-order rate constants determined from the data for 106 deletion, 106 Val, 106 Leu, and 106 Asp are of the same order of magnitude as the rate for the direct reduction of P-450$_{cam}$ by methylviologen radicals.[11] The slow apparent second-order rate for the 106 Lys variant could be explained if the reduction of methylviologen by reduced putidaredoxin is the rate-limiting step. On the basis of this analysis, much of the residual rate of P-450$_{cam}$ reduction measured in the presence of nonaromatic variant putidaredoxins comes from direct reduction by methylviologen radicals.

The exact lower rate limits for the electron transfer catalyzed by the nonaromatic variants are not yet known. Nonetheless, their diminished activity compared to that of the aromatic C-terminal putidaredoxins is striking. In general, mutational analysis is complicated by potential conformational changes such as those seen in the Ser 82 mutant of cytochrome *c* which confound simple cause-and-effect relationships.[12] Because of the near identity in physical properties of putidaredoxin's iron–sulfur center and its association with P-450$_{cam}$, large conformational changes in these putidaredoxin variants seem unlikely. It is also known that the spectral distribution of protein motion could alter reorganizational energies that couple to electron-transfer events.[13] Such dynamics

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could be changed by mutation, and hence, the presence of a C-terminal aromatic residue may potentiate a protein conformation that is favorable for electron transfer but that is not reflected in the equilibrium binding energy. A more exciting possibility is that an extended π-electron system at putidaredoxin's C-terminus could serve as a mediator of the electron-transfer event. A similar role for aromatic mediation has been suggested for phenylalanine 82 in the cytochrome *c*/cytochrome *c* peroxidase system.[2e,g,i]

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Azulenic Retinoids and the Corresponding Bacteriorhodopsin Analogues. Unusually Red-Shifted Pigments

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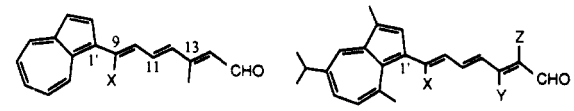
There has been extensive effort directed toward a better understanding of the red-shifted UV-vis absorption characteristics of visual pigments and bacteriorhodopsin (BR) analogues as distinguished from the absorption characteristics of the protonated Schiff bases (PSB). The proximity of a second point charge originating from the protein is believed to be important[1] although the medium and the extent of protonation are also suspected to have a significant effect on pigment absorption properties.[2] Less successful has been the search for extensively red-shifted pigment analogues. For rhodopsin, the most red-shifted analogue known is the 13-CF_3 analogue (545 nm),[3] and for BR, the most red-shifted analogues known are the cyanine dye analogues (662 nm)[4] and a minor equilibrium concentration of the 14-F analogue (680 nm).[5] We now report the preparation of BR analogues with long-wavelength absorption maxima beyond 750 nm.

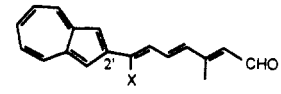
Azulene and guaiazulene retinoids **1–6** have been synthesized by following established procedures for olefination of the known acylated azulenes.[6] The methods gave mixtures of 13-cis and all-trans isomers which were separated by preparative HPLC and characterized by ^{1}H NMR spectroscopy.

While all these azulene retinal analogues were found to interact readily with bacterioopsin (BO), the absorption properties of the resultant pigments varied considerably with the long-wavelength band ranging from 520 (**2**) to 830 nm (**5**). Data are listed in Table I along with those of the protonated Schiff bases. As an example,



1 : X = H
2 : X = CF_3

3 : X = H, Y = CH_3, Z = H
4 : X = CF_3, Y = CH_3, Z = H
5 : X = H, Y = CF_3, Z = H
6 : X = H, Y = CH_3, Z = F



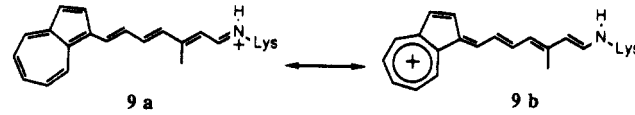
7 : X = H
8 : X = CH_3

spectra taken during formation of the 795-nm pigment of **6** are shown in Figure 1.

Two azulene-containing analogues (**7** and **8**) are known,[7] with the side chain originating from the 2'- rather than 1'-position as in **1–6**. The BR analogue of **7** has a long-wavelength absorption band centered at 475 nm[7a] while the phototaxis action spectrum of **8** in the algae *Chlamydomonas* centered at 472 nm.[7b] The lack of substantial red shifts is in complete contrast with the present data.

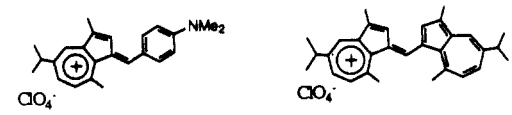
We note that the observed difference for the 1'- and 2'-substituted azulene analogues cannot be due to relative ordering of the two lowest azulene excited states that account for their unique colors. Symmetry consideration led to the conclusion that the 2'-substituted analogues would cause a red shift of the allowed S_2 band and the opposite for the 1'-substituted analogues.[8] This trend is contrary to the observed results. Neither would the large difference between the 1-substituted (to >800 nm) and the 2'-substituted azulenes (475 nm) likely be due to previously known factors alone, such as altered relative position of the second point charge. Instead, we offer the following additional explanation for the unusual red-shifted characteristics of several of the 1'-substituted azulene analogues.

The resonance hybrid of the protonated retinyl Schiff base can be represented by the imino (**9a**) and the enamino (**9b**) resonance structures. Because of the stability of the tropylium ion, we believe

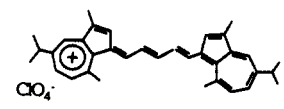


9 a **9 b**

that, in the 1'-substituted azulene BR analogues, the enamino resonance structure should dominate, giving rise to the unusual red-shifted absorption characteristics. Consistent with this explanation are the absorption maxima reported for the following conjugated tropylium ions.[9]



647 (logε 4.98) **680** (5.08)



860 (5.49)

The seemingly disparate absorption properties of the above BR analogues can be rationalized by the relative importance of the two resonance contributing structures. The electron-donating alkyl

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