

Table I. Rate Parameters^a for the Oxidation of Dihyronicotinamides by 7-Acetylflavo-GAPDH and 7-Acetyl-10-methylisalloxazine

substrate	7-AcFl-GAPDH, (k_{cat}/K_m) _{app} M ⁻¹ s ⁻¹	7-AcFl, k_2 , M ⁻¹ s ⁻¹	(k_{cat}/K_m) _{app} / k_2
NADH	1067 ± 31	12.9 ± 0.3	83
NADPH	57 ± 5	21.7 ± 1.4	2.6
PNAH ^b	624 ± 61	948 ± 14	0.65
BNAH ^b	487 ± 32	181 ± 7	2.7

^a Determined at 340 nm in air-saturated buffer (50 mM EPPS, pH 8.0, 25 °C) by method of initial rates; [flavin] = 3.71×10^{-7} M; [superoxide dismutase] = 0.01 mg/mL; [catalase] = 0.086 mg/mL. ^b PNAH and BNAH refer to *N*-propyl- and *N*-benzyl-1,4-dihydro-nicotinamide, respectively.

Table II. Rate Parameters^a and Product Ratios¹⁴ for the Oxidation of NADH and Selectively Deuterated NADH Derivatives Catalyzed by 7-Acetylflavo-GAPDH and 7-Acetylflavin

substrate	7-AcFl-GAPDH		7-AcFl	
	(k_{cat}/K_m) _{app} , M ⁻¹ s ⁻¹	(4-D)NAD ⁺ / (4-H)NAD ⁺	k_2 , M ⁻¹ s ⁻¹	(4-D)NAD ⁺ / (4-H)NAD ⁺
NADH	1067		13.4	
(4 <i>R</i> -D)NADH	905	13.3	4.34	1.86
(4 <i>S</i> -D)NADH	224	1.17	9.31	10.8
(4-D ₂)NADH	113		2.02	

^a Conditions as described in Table I.

preference for hydrogen transfer from the *si*-face of NADH in the enzymatic reaction and to elucidate the factors affecting the magnitude of the primary kinetic isotope effect.

In summary, we have developed a new series of semisynthetic flavoproteins based on GAPDH, a tetrameric protein template. In addition to rate accelerations of at least 120-fold for the oxidation of NADH, flavo-GAPDH's exhibit predictable substrate specificity and stereoselectivity opposite that of our earlier flavopapains. Our initial successes now make possible the preparation of more sophisticated artificial enzymes that take advantage of the tetrameric structure of the GAPDH template.

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Sterically Hindered Triplet Energy Transfer

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For two decades now triplet energy transfer has played a key role in mechanistic investigations of photochemistry.⁴ The usefulness of Stern-Volmer quenching studies in determining triplet lifetimes relies on accurate knowledge of energy-transfer rate constants. It is well established that *exothermic* bimolecular triplet energy transfer generally proceeds with rate constants that are close to diffusion controlled pretty much independent of donor and acceptor structure.⁴ Several studies have found either small⁵

Table I. Representative Rate Constants for Quenching Ketones by Triplet Energy Transfer^a

ketone	solvent	quencher	k_q , 10 ⁹ M ⁻¹ s ⁻¹
PhCOCH ₃	benzene	1-MN ^b	8.4
xanthone	methanol	1-MN	9.3
<i>p</i> -MeOPhCOCH ₃	benzene	dMH ^c	5.9
<i>p</i> -MeOPhCOCH ₃	methanol	dMH	8.3
<i>p</i> -MeOPhCOt-Bu	benzene	dMH	5.0
<i>p</i> -MeOPhCOt-Bu	methanol	dMH	6.2
2-MeOPhCOPh	C ₆ H ₅ Cl	dMH	4.8
2-MeOPhCOPh	methanol	dMH	9.2
2-BzOPhCOPh	C ₆ H ₅ Cl	dMH	2.1
2-BzOPhCOPh	methanol	dMH	5.9
2,6-(MeO) ₂ PhCOPh	C ₆ H ₅ Cl	dMH	2.8
2,6-(MeO) ₂ PhCOPh	methanol	dMH	5.4
2,2'-(BzO) ₂ PhCOPh	C ₆ H ₅ Cl	dMH	0.8
phenanthrene	methanol	dMH	5.8
paracyclophane	benzene	dMH	2.8
PhCOCH ₃	C ₆ H ₅ Cl	dMH	5.0
PhCOCH ₃	C ₆ H ₅ Cl	COD ^d	2.0
KPC	methanol	dMH	2.3
KPC	C ₆ H ₅ Cl	dMH	0.88
KPC	methanol	COD	0.19
KPC	C ₆ H ₅ Cl	COD	0.08
xanthone	methanol	biphenyl	8.8
PhCOC ₃ H ₇	benzene	biphenyl	2.5 ^e
Ph ₂ CO	benzene	biphenyl	0.3 ^e
KPC	benzene	biphenyl	0.0008

^a Measured at room temperature; standard deviations typically ±5%. ^b 1-Methylnaphthalene. ^c 2,5-Dimethyl-2,4-hexadiene. ^d 1,3-Cyclooctadiene. ^e Reference 15.

or negligible⁶ steric effects on this process. One of us has pointed out that energy transfer generally is so rapid at van der Waals separation of donor and acceptor molecules as to preclude significant steric effects.⁶ We now wish to report some examples of significant steric effects on exothermic triplet energy transfer, examples that better define the geometric requirements of this process.

We have measured rate constants for quenching of various triplet aryl ketones by conjugated dienes and some aromatics. The technique involves measurement of the triplet decay rate as a function of quencher concentration and in some cases verification by measuring sensitized triplet naphthalene absorption.⁷ Table I summarizes the most pertinent results.

For a wide variety of phenyl ketones not substituted in either the ortho or α positions, rate constants at 27 °C cover the narrow ranges of (6–8) × 10⁹ M⁻¹ s⁻¹ in benzene and (8–11) × 10⁹ M⁻¹ s⁻¹ in methanol. Rate constants for quenching *p*-methoxy-pivalophenone⁸ are 75% as large as for quenching *p*-methoxyacetophenone. This verifies our earlier conclusion that the effects of α -dimethyl substitution are minimal.⁶ Likewise, 2,6-dimethoxy substitution results in only a halving of the rate constant.

Several *o*-benzyloxy ketones show larger effects. Thus each ortho benzyloxy group on benzophenone decreases k_q by a factor of 0.4. Since the rate constants for the ortho-substituted ketones are still partially diffusion controlled, we emphasize that the larger ortho groups indeed produce significant decreases in the rate for energy transfer between nearest-neighbor molecules. These decreases are disguised by the diffusion control.⁶ For example, various workers have estimated the actual in-cage energy-transfer rate k_{et} as (5–10) × 10¹⁰ s⁻¹.^{9,10} With a comparable rate for diffusion apart of donor and acceptor, observed rate constants in

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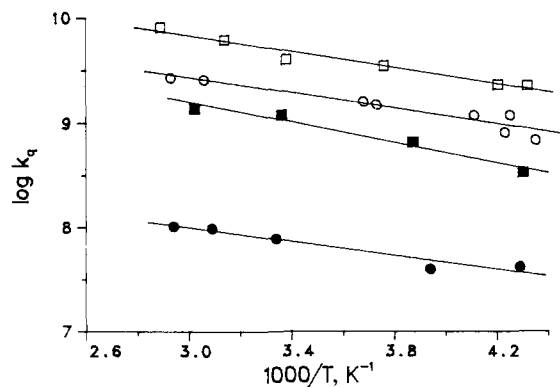
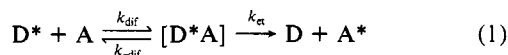


Figure 1. Arrhenius plots for the quenching by dienes of ketone triplet-triplet absorption in chlorobenzene: (□) acetophenone and dMH ($A = 10^{11.0}$); (○) acetophenone and COD ($A = 10^{10.5}$); (■) KPC and dMH ($A = 10^{10.0}$); (●) KPC and COD ($A = 10^{9.0}$).

light solvents are typically about half that for diffusion.¹⁰ Cutting the observed rate constant in half represents a factor of 4 decrease in k_{et} itself. These steric effects would not be detectable at all if n, π^* triplets were not somewhat inefficient energy donors.^{6,10}



We prepared ketoparacyclophane (KPC) to see how the orthogonal arrangement of carbonyl and aromatic π -systems would affect energy transfer. In glasses at 77 K KPC shows a 0-0 phosphorescence band at 69 kcal/mol, as Cram found.¹¹ The almost featureless nature and 20-ms lifetime of the emission suggests a π, π^* lowest triplet. This conclusion is reinforced by the >2 - μ s lifetime of triplet KPC in cyclopentane, where n, π^* ketone triplets react in less than 0.2 μ s.¹² Energy-transfer rate constants in methanol and chlorobenzene are $1/4$ and $1/6$, respectively, the values for acetophenone. In contrast, triplet paracyclophane and triplet phenanthrene, which have the same π, π^* excitation energy, are quenched by dimethylhexadiene at much the same rate. We judge the relatively slow energy-transfer rate constants for KPC to reflect stereoelectronic factors rather than steric hindrance. The n orbital is less shielded than in any other phenyl ketone, whereas the carbonyl π -orbital is relatively more shielded than usual by the ortho hydrogens. Since the lowest triplet is π, π^* , the orthogonality of the phenyl and carbonyl π -systems apparently inhibits proper orbital overlap. It has been suggested already that energy-transfer rate constants are affected by the ease with which the orbitals involved can develop sufficient overlap.^{10,13}

It has been known for some time that 1,3-cyclooctadiene¹⁴ and biphenyl¹⁵ are sufficiently twisted to be slower quenchers than are planar dienes and aromatics. The table points out that both compounds quench nonhindered triplet ketones with rate constants $\sim 1/3$ as large as the maximum values observed in a given solvent. Toward KPC the difference is a factor of 10 for cyclooctadiene. Figure 1 compares Arrhenius plots for diene quenching in chlorobenzene. The observed E_a values are all 1.6 ± 0.4 kcal. Remarkably, the differences in rates involve mainly entropy effects, as enumerated in the figure caption. This observation means that the rate decreases accompanying twisted acceptor π -systems do not involve the acceptors' higher triplet energies. Rather, at van der Waals approach distances there must be fewer accessible orientations that allow the orbital overlap necessary for energy transfer. Current models for energy transfer that assume negligible

entropic effects¹⁶ may require reevaluation.

The behavior of biphenyl further dramatizes this stereoelectronic effect. With xanthone (π, π^*) and acetophenone (n, π^*) as donors, energy transfer is several kilocalories per mole exothermic and k_q is relatively large, close to the maximum solution value for the former. With benzophenone and KPC as donors, energy transfer is thermoneutral,¹⁵ and the combination of twisted donor and acceptor results in a 1000-fold decrease in k_q , comparable to that for acetone-acetone energy transfer.¹⁷

We feel that these results combined with scattered literature reports allow two important general conclusions. Steric effects that limit closeness of approach are relatively unimportant in triplet energy transfer, primarily because diffusion control masks small but real steric decreases in in-cage energy-transfer rates. However, large stereoelectronic effects appear when the π -systems of donor or acceptor are twisted such that the orbital overlap necessary for electron exchange is minimized.¹⁸ In such cases, the built-in inefficiency already noted for n, π^* triplets^{10,13,17} takes over.

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Photodisproportionation of (4-Acylpyridine)tungsten(0) Pentacarbonyl Complexes

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We wish to report an overlooked photoreaction of the much-studied $W(CO)_5(\text{pyridine})$ complexes.¹⁻⁸ Irradiation of these complexes in the presence of CO, alcohol, or alkene causes efficient replacement of the pyridine by added ligand.³⁻⁸ In the presence of excess pyridine, $W(CO)_4(\text{pyr})_2$ formation occurs in low efficiency.^{2-4,6} This competing reaction has been attributed to CO cleavage competing with pyridine cleavage.^{2-4,6} We chose to do the irradiations in nonnucleophilic hydrocarbon solvents, under which conditions a clean, hitherto unreported disproportionation to $W(CO)_6$ and $W(CO)_4(\text{pyr})_2$ occurs.⁹ Unexpectedly, the reaction caused by visible light involves dissociation of pyridine rather than CO and subsequent attack of $W(CO)_5$ on ground-state reactant.

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