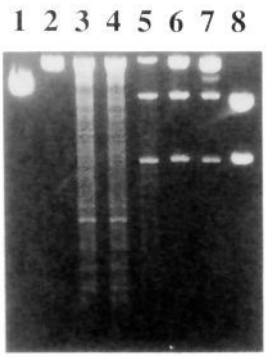

Figure 2. A 1% agarose gel stained by ethidium bromide. Lane 1: supercoiled plasmid pUC19-O_R1 DNA. Lane 2: plasmid DNA linearized by *Sca*I. Lane 3: *Sca*I-linearized plasmid DNA (3 μg, 0.18 μM) treated with free K84C staphylococcal nuclease (0.8 μM). Lane 4: same as in lane 3 but in the presence of monomeric repressor (1.0 μM). Lane 5: *Sca*I-linearized DNA (2 μg, 0.12 μM) treated with the hybrid protein (0.2 μM). Lane 6: same as in lane 5 but in the presence of poly(A) (10 μg). Lane 7: supercoiled plasmid DNA (3 μg, 0.18 μM) treated first with the hybrid protein (0.13 μM) in the presence of poly(A) (10 μg) and then digested with *Sca*I. Lane 8: *Sca*I/*Hin*dIII digest of pUC19-O_R1. Cleavage reactions were performed by mixing the plasmid pUC19-O_R1 (2–3 μg), poly(A) as indicated, and enzyme (0.1–0.8 μM) in 10 mM PIPES, pH 7.0, 0.1 mM EDTA, and 40 mM NaCl, in a total volume of 10 μL. After incubation for 20 min at room temperature, the reaction was initiated by the addition of $CaCl_2$ to a final concentration of 10 mM and terminated after 15 s by the addition of ethylene glycol bis(1-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) to a final concentration of 12 mM.

In order to analyze the ability of the hybrid protein to selectively hydrolyze duplex DNA, a DNA fragment containing the O_R1 repressor binding site (Figure 1) was chemically synthesized and inserted into the *Acc*I/*Hin*dIII site of plasmid pUC19.[16] The resulting plasmid pUC19-O_R1 (2704 bp) contained the 17-base-pair repressor binding site, O_R1, with A,T-rich sites d(T_4A_4) on each side.[17] The supercoiled plasmid DNA (3 μg, 0.18 μM, 500 μM in base pairs) was preincubated with stoichiometric amounts of the hybrid protein, and the cleavage reaction was then initiated by the addition of Ca^{2+} (the nuclease is Ca^{2+}-dependent[18]). The reaction was quenched, and the product was then treated with the restriction enzyme *Sca*I to generate discrete fragments, which were analyzed by agarose gel electrophoresis. Comparison of these fragments with known standards (1730 and 974 bp, generated by *Sca*I/*Hin*dIII[19] digestion of pUC19-O_R1) indicated that the cleavage was highly specific and occurred adjacent to the O_R1 site (Figures 1 and 2).

In a similar experiment, the plasmid DNA was first linearized with *Sca*I and then treated with the hybrid protein. The linear DNA (2 μg, 0.12 μM, 330 μM in base pairs) was also efficiently hydrolyzed (approximately 50% conversion) at the target site (Figure 2). Another plasmid, pLcIIFXβ[20] (approximately 3000 bp), which contains the λ operon but not the eight-base-pair A,T-rich sites, was also selectively cleaved adjacent to the O_L1 sequence. Control experiments showed that neither K84C staphylococcal nuclease alone nor free nuclease in combination with repressor was able to selectively cleave the target sequence. Specific cleavage was accompanied by some nonspecific hydrolysis, presumably by unbound hybrid protein. Addition of poly(A) effectively depressed this nonspecific cleavage. Experiments are currently being carried out to determine the nature of the double strand cleavage reaction.

We have demonstrated that a combination of chemical and genetic modifications can be used to convert a relatively nonspecific enzyme into a sequence-selective DNA-cleaving molecule. This strategy should be applicable to other DNA-binding proteins and may lead to a family of hybrid nucleases capable of selective cleavage of large DNAs.

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cis-Stilbene Fluorescence in Solution. Adiabatic $^1c^* \rightarrow {}^1t^*$ Conversion

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Though *cis*-stilbene fluorescence has been observed in high-viscosity media,[1-5] starting with the important contribution by Lewis and co-workers,[6] all previous attempts to detect its fluorescence in fluid solution have failed. Recently, however, fluorescence with an extremely long lifetime, 20 ns, was reported from supersonic beams of *cis*-stilbene vapor seeded in inert gas expansions and was attributed to trapping of vibrationally relaxed $^1c^*$ in an inherent minimum on the S_1 potential energy surface.[7] This interpretation sets an upper limit of $k_f \leq 5 \times 10^7$ s^{-1} for the radiative rate constant of $^1c^*$ that is nearly one-third the value based on the 4.7-ns lifetime of $^1c^*$ measured at 77 K in 3-methylpentane glass[8] under conditions for which values of ϕ_f = 0.75–0.79 have been reported.[3,4] An earlier time-resolved investigation of *cis*-stilbene vapor, in which transient decay was monitored by multiphoton ionization,[9] reported single-exponential decay, τ = 0.32 ps, that was associated with motion along the torsional coordinate of $^1c^*$ unimpeded by any appreciable barrier.[9] Somewhat longer lifetimes, τ = 0.9–1.35 ps and 1.0 ps, have been obtained by monitoring $^1c^*$ absorption[10-12] and $^1c^*$ fluorescence,[13] respectively, in *n*-hexane solution at room temperature, suggesting a small medium-imposed torsional barrier.[14]

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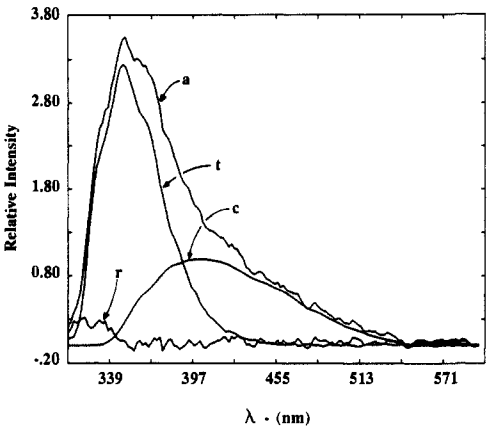

Figure 1. Average of 10 pure base-line-corrected *cis*-stilbene experimental emission spectra, a, together with PCA-SM pure-component stilbene solution spectra, trans, t, and cis, c. The cis spectrum has been smoothed to remove experimental noise. Subtraction of the pure-component spectra from the experimental spectrum gives the residual curve r. All spectra are uncorrected for instrumental nonlinearity.

In this work, highly purified *cis*- and *trans*-stilbene-d_0 and -d_2 were obtained by repeated conversions from trans to cis (fluorenone-sensitized photoisomerization) and cis to trans (iodine catalysis) with careful purification by alumina chromatography (*n*-pentane eluent) at each step, followed by distillation of the cis and sublimation of the trans isomer.[15] Fluorescence measurements were carried out at 30.0 °C in Fisher HPLC grade *n*-hexane. Spectra were measured with a modified Perkin-Elmer MPF-2A fluorometer with operation and data acquisition controlled by a Dell Corporation 80286/87 (12 MHz) microcomputer. To minimize cis → trans photoisomerization in the course of the measurements, 500-mL solutions were circulated from a reservoir through the 4-mL cell compartment. Under these conditions, a gradual increase in trans concentration was reflected in small systematic increases in the trans portion of the fluorescence spectrum (0.87% per spectrum). After correction for this trans buildup, a significant contribution of *trans*-stilbene fluorescence (51-54% of total area) remained, even from the purest *cis*-stilbene solutions (99.98% cis, 0.021% trans, by GLC), Figure 1. The *cis*-stilbene fluorescence spectrum was resolved by use of principal-component-self-modeling analysis (PCA-SM)[16] on a matrix consisting of 76 spectra, $310 \leq \lambda_{em} \leq 596$ nm, from *n*-hexane solutions containing 2.79×10^{-5} M *cis*-stilbene and a series of added *trans*-stilbene concentrations in the range $0 \leq$ [*trans*-stilbene] $\leq 1.16 \times 10^{-7}$ M. Excitation wavelengths were 268.0, 269.6, and 272.0 nm. PCA-SM analysis yielded a structureless spectrum for *cis*-stilbene, $\lambda_{max} \simeq 408$ nm (corrected for nonlinearity of instrumental response), somewhat blue-shifted from spectra obtained in rigid media and low *T*.[1-5] The spectrum contained a small peak at ~320 nm that decreased in size with repeated purification of *cis*-stilbene but could not be entirely eliminated (~3% area in the final spectrum). It was artificially removed by assuming Gaussian shape at the onset of $^1c^*$ emission, Figure 1. Using 9,10-diphenylanthracene as standard, $\phi_f = 0.90 \pm 0.04$,[17,18] at $\lambda_{exc} = 269.6$ nm, the quantum yield of the *cis*-stilbene portion of the spectrum, corrected for instrumental nonlinearity, is $(8.9 \pm 0.7) \times 10^{-5}$. Use of the Strickler-Berg equation[19,20] gives $k_f = (1.4 \pm 0.1) \times 10^8$ s^{-1} for $^1c^*$ in *n*-hexane at 30 °C, which is about 40% larger than predicted by the transient lifetimes and our quantum yield. Deuteration at the vinyl positions increases ϕ_f for the trans isomer, $\phi_{fd2}/\phi_{fd0} = 1.54 \pm 0.04$, in agreement with the fluorescence lifetime ratio,[21] but does not affect ϕ_f for the cis isomer $\phi_{fd2}/\phi_{fd0} = 1.01 \pm 0.10$, consistent with the absence of a significant inherent barrier in $^1c^* \rightarrow {}^1p^*$ torsional motion.[22] By analogy with *trans*-1-phenylcyclohexene,[23] we assume that the source of the deuterium isotope effect in the trans isomer is loss of the zero-point energy of the out-of-plane olefinic CH vibration in passing over the inherent torsional barrier.

Use of neutral density filters to attenuate the intensity of the excitation beam by 41%, 53%, and 63% was shown to affect trans/cis contribution ratios in the fluorescence spectra only slightly, eliminating the possibility that a significant portion of $^1t^*$ formation requires absorption of two photons, the first of which produces ground-state trans. This conclusion was confirmed by excitation spectra obtained by monitoring fluorescence from pure cis solutions at 404 nm (~70% cis contribution) and at 350 nm (~90% trans contribution). The resulting spectra are very similar and differ substantially from the excitation spectrum of the pure trans solution, monitored at 350 nm; the latter shows the expected red shift of λ_{max} and the characteristic structure of the absorption spectrum of the trans isomer. After correction for the contribution of the small trans impurity and of the maximum amount of trans isomer that may form from $^1c^*$ in the path of the exciting beam, we conclude that no less than 72% of the trans fluorescence observed from our cis solutions is a direct result of one-photon excitation of $^1c^*$. This remarkable conclusion means that 0.16% of $^1c^*$ molecules avoid or escape nearby excited-state potential energy minima at the perpendicular geometry or at dihydrophenanthrene and undergo adiabatic $^1c^* \rightarrow {}^1t^*$ conversion giving rise to $^1t^*$ fluorescence.[24] Experiments at different temperatures and in different media are in progress.

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Luminescence Line Narrowing: A Highly Specific Probe of Excited States in Rhodium(III) Mixed-Chelate Complexes

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The properties of the lowest excited states of d^6 chelate complexes continue to attract a great deal of interest.[1] Numerous physical and chemical techniques have been used to probe the nature of these excited states. Luminescence line narrowing (LLN) is not among them,[2] and in the present communication we demonstrate the power of this technique when applied to mixed-chelate complexes of Rh^{3+}. It turns out to be highly specific with regard to the ligand involved in the transition. By combining

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