linked PQ system[9] and comparable to the value observed for CR in rigid non-PQ models.

In summary, ET rates for the homologous PQ models **4a–d** have been established both by steady state fluorescence and transient spectral studies. Spectroscopic identification of the P^+Q^- transients definitively relates diminished $^1P^*$ lifetimes and fluorescence yields to ET and also affords the CR rate of P^+Q^- to PQ. Observed β values show that the distance and σ-bond dependence of ET in rigidly linked PQ and non-PQ systems can be comparable. The observed and theoretically predicted abilities of these three PQ linkers to promote ET diminish the order spirocycles > bicyclo[2.2.2]octanes > alkanes. This result supports the view that long-range ET occurs through bonds.[5,7,44-46]

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Photoregulation of α-Chymotrypsin by Its Immobilization in a Photochromic Azobenzene Copolymer

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Photoregulation of enzymes is of broad interest as a means for developing macromolecular light signal amplification devices and targeted therapeutic agents. In previous studies[1-8] photoregulation of the biocatalyst was made possible by chemical modification of either the enzyme's active site or the protein backbone with photochromic compounds. Another approach involved the application of photochromic inhibitors, acting as effectively in only one of the photochromic isomers. Nevertheless, none of the known examples exhibits complete "on-off" photoswitchable activity. Here we report on a novel approach for photostimulation of enzymes by their immobilization in photochromic polymer matrices. We find that immobilization of α-chymotrypsin in a cross-linked photochromic copolymer of acrylamide and 4-(methacryloylamino)azobenzene (**1**) leads to complete "on-off" photoregulation of the enzyme, at a certain composition of the copolymer matrix. Previous studies have emphasized photoregulated physical properties of photochromic polymer assemblies such as viscosity,[9-11] wettability,[12] sol-gel transition,[13] electric potential,[14] and size changes.[15] The present study highlights the application of photochromic polymers as reaction media for photoregulation of biocatalysts.

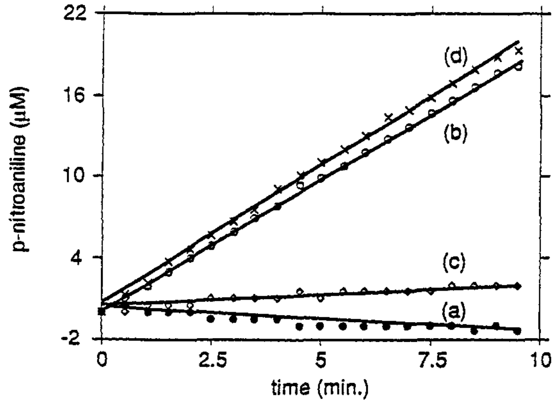
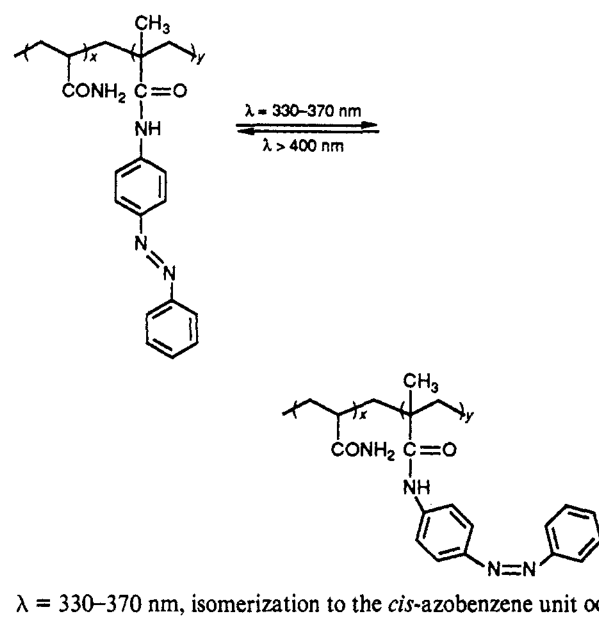


Figure 1. Rate of hydrolysis of **2**, 5.7×10^{-3} M, by α-chymotrypsin, 47.6 units, immobilized in an acrylamide–**1** copolymer (0.5 mol %): (a) hydrolysis by the enzyme in *trans*-**1**–acrylamide copolymer form; (b) after illumination, λ = 330–370 nm and in the presence of *cis*-**1**–acrylamide copolymer, (c) after further illumination of the polymer, λ > 400 nm, and re-formation of *trans*-**1**–acrylamide; (d) after additional illumination, λ = 330–370 nm, and regeneration of *cis*-**1**–acrylamide. Before each run the polymer gel is washed with TEA buffer (pH = 7.8) and new substrate solution is introduced to maintain similar initial concentrations of the substrate.

Immobilization of α-chymotrypsin (E.C. 3.4.21.1, 585 units) in the photochromic polymer is accomplished by radical copolymerization of acrylamide (375 mg) and **1**, 0–1 mol %, using *N,N'*-methylenebis(acrylamide) (20 mg) as a cross-linker in the presence of the enzyme. The resulting polymer gel that includes the enzyme is thoroughly washed. The polymer contains *trans*-azobenzene components and exhibits reversible photochromic properties (eq 1). Upon illumination of the polymer assembly,



(1)

λ = 330–370 nm, isomerization to the *cis*-azobenzene unit occurs.

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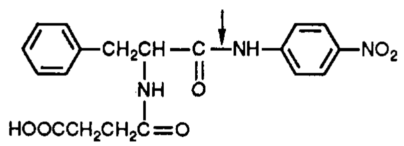
Table I. Normalized Rates of Hydrolysis of **2** by α-Chymotrypsin Immobilized in the Photochromic Copolymer at Different Degrees of Loading[a]

	load (mol %) =				
	0.18	0.3	0.5	0.75	1
v_{cis}	5	4	4	1	1
v_{trans}	2	1	0–0.3	1	1

[a] A value of 1 corresponds to a rate of 0.5 μM min^{-1} of *p*-nitroaniline formation.

In fact, a photostationary cis/trans equilibrium corresponding to 1.04 is obtained. Illumination of the resulting *cis*-azobenzene polymer, λ > 400 nm, restores almost entirely the *trans*-azobenzene polymer.

Photoregulation of α-chymotrypsin is studied by cutting slices of the photochromic polymer, ca. 2 mm wide, that include 47.6 units of immobilized enzyme and following the rate of hydrolysis of *N*-(3-carboxypropionyl)-L-phenylalanine *p*-nitroanilide[16] (**2**), 5.7 × 10^{-3} M, by the immobilized enzyme. Figure 1 shows the



2

cyclic photoregulation of the biocatalyst immobilized in a polymer that includes 0.5 mol % of the photochromic component in the copolymer structure: No hydrolysis of **2** occurs by the enzyme entrapped in the *trans*-azobenzene copolymer, Figure 1a. Photoisomerization of the copolymer to *cis*-azobenzene results in a biocatalytic assembly that effectively hydrolyzes the substrate at a rate of 2 μM min^{-1}, Figure 1b. Further illumination of the polymer–enzyme assembly, λ > 400 nm, restores the *trans*-azobenzene polymer, and the enzyme is again deactivated, Figure 1c. Additional illumination, λ = 330–370 nm, of the copolymer and production of the *cis*-azobenzene polymer restores the biocatalytic activity of the assembly, Figure 1d. It is evident that the biocatalytic transformation is completely and reversibly "on-off" switchable in the photochromic copolymer–enzyme assembly. Control experiments reveal that the activity of α-chymotrypsin in the *cis*-azobenzene copolymer is ca. 2-fold faster than the activity of the enzyme in a pure acrylamide gel. The switching efficiency of immobilized α-chymotrypsin and its activity strongly depend on the loading degree of the polymer by the photochromic material, Table I. It is evident that, at low loading degrees of the copolymer by the photochromic material, incomplete switching of the biocatalytic assembly is obtained, and as the loading degree increases up to a value of 0.5%, the activity of the enzyme in the *trans*-azobenzene copolymer structure declines. At 0.5% loading, complete switchable activity of the biocatalyst is observed. Further increase of the loading decreases the biocatalyst performance in both photochromic forms of the polymer.

The photostimulated activity of the enzyme in the functionalized polymer could originate from structural changes of the protein backbone induced by the volume changes of the polymer. Alternatively, photoregulation of the permeabilities of the polymer backbone[17] toward the substrate (**2**) might photoregulate the entrapped biocatalyst.

Gel filtration and flow dialysis experiments reveal that *trans*- and *cis*-**1**-acrylamide copolymers differ substantially in their permeabilities toward the substrate **2**. We find that *cis*-**1**–acrylamide is permeable toward the substrate, while *trans*-**1**–acrylamide is essentially nonpermeable toward it. We thus conclude that photoregulation of α-chymotrypsin immobilized in the photochromic azobenzene copolymer is controlled by the permeability of the substrate across the polymer matrix. Application of other photochromic polymers and immobilization of different enzymes are being further examined as photoregulated biocatalytic assemblies.

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Partial Oxidation of Olefins by Molecular Oxygen Catalyzed by (Alumina)Rh(O_2)

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Direct, partial oxidation of an olefinic double bond by molecular oxygen, catalyzed by a metal complex, is rare,[1] but we have now found a novel example: oxide-bound species (alumina)Rh(O_2)[2] efficiently catalyzes reaction between O_2 and olefins to give ketones. In this procedure, no "sacrificial" ligands are required,[3] and under our conditions, little combustion or double-bond cleavage[4] is noted. Thus, this new reaction is fundamentally different from those based on soluble Rh(O_2) complexes,[3,4] including organic oxygen-ligated (acac)Rh(O_2),[4] and product distributions and relative olefin reactivities show it to be unlike Wacker[1c] or radical[5] oxidation pathways.

The catalyst (alumina)Rh(O_2) was prepared[2,6] by reaction between Rh(allyl)$_3$ and alumina followed by carbonylation[7] and O_2 treatment.[2] When a stream of O_2 (ca. 1 atm) was passed through a reservoir of cyclohexene and then over a frit charged with 100 mg of (alumina)RhO_2 (5.6 μmol of Rh) at 280 °C for 4 h, cyclohexanone (6.0 mg; 61 μmol; 2.7 equiv (equiv of Rh)$^{-1}$ h^{-1}) was collected in a cold trap (−78 °C); no products of allylic attack were obtained, suggesting that radical pathways are not important with this system.[5] Similarly, when a stream of O_2 was passed first through norbornene[8] and then over the catalyst, GC analysis showed that 2-norbornanone (416 mg; 3.8 mmol; 168 equiv (equiv of Rh)$^{-1}$ h^{-1}) and cyclohexene-4-carboxaldehyde (178 mg; 1.6 mmol; 72 equiv (equiv of Rh)$^{-1}$ h^{-1}) were produced,[9] and 2,3-dimethyl-2-butene gave pinacolone (437 mg; 4.4 mmol; 260 equiv (equiv of Rh)$^{-1}$ h^{-1}).[10] These observed skeletal rear-

(1) (a) The outstanding example of an O_2-based process is silver-catalyzed ethylene oxidation to ethylene oxide. See, for example: Lee, J. K.; Verykios, X. E.; Pitchai, R. *Appl. Catal.* **1989**, *50*, 171. Sarjkowski, D. J.; Boudart, M. *Catal. Rev. Sci. Eng.* **1987**, *29*, 325. (b) For another example of direct O_2 involvement, see: Groves, J. T.; Quinn, R. *J. Am. Chem. Soc.* **1985**, *107*, 5790. Marchon, J.-C.; Ramasseul, R. *J. Chem. Soc., Chem. Commun.* **1988**, 298. (c) Our process stands in contrast to "Wacker" oxidation in which oxygen acts as an *indirect* oxidizing agent which reoxidizes the used catalyst. See: Henry, P. M. *J. Am. Chem. Soc.* **1966**, *88*, 1595.

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(6) For example, 30 mg of Rh(allyl)$_3$ was treated with a slurry of 1 g of Degussa aluminum oxide C (100 m^2 g^{-1}; dried at 250 °C) in toluene at room temperature for 96 h. After removal of the toluene, 1 atm of CO was passed over the resulting powder at room temperature for 12 h,[7] followed by treatment with dry O_2 (1 atm) at 140 °C for 170 h.[2] The resulting catalyst was 1.1% Rh by weight.

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(9) No oxidation was observed catalyzed by the support in the absence of the Rh complex.

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