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Supporting Information

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Photochemical Regulation of Restriction Endonuclease Activity

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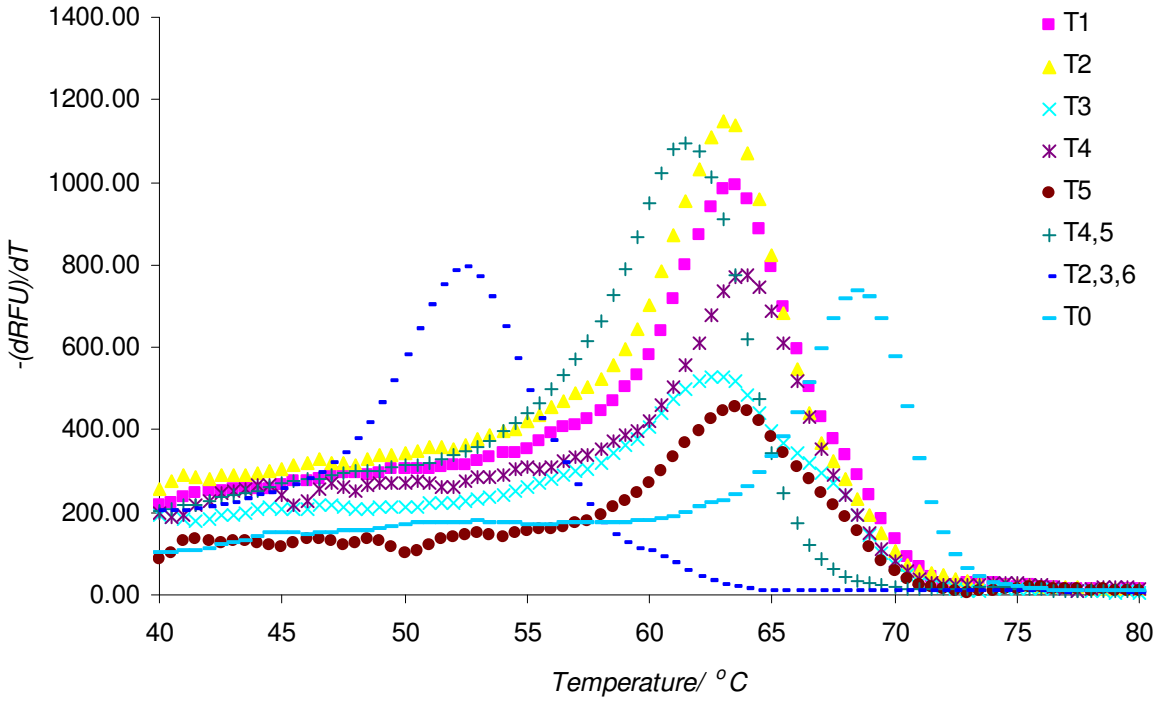
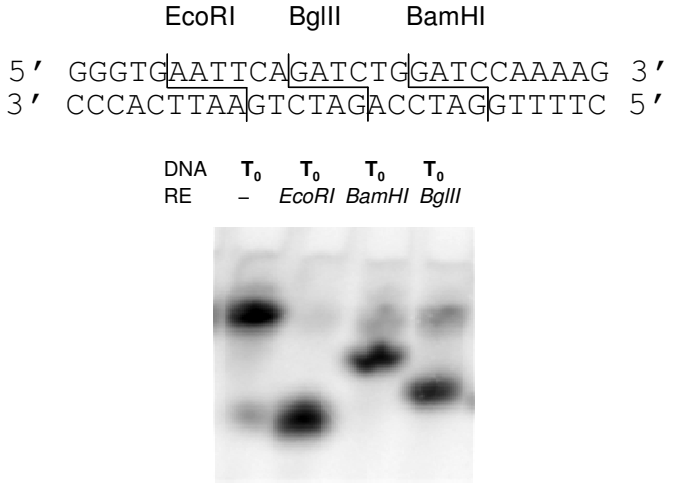
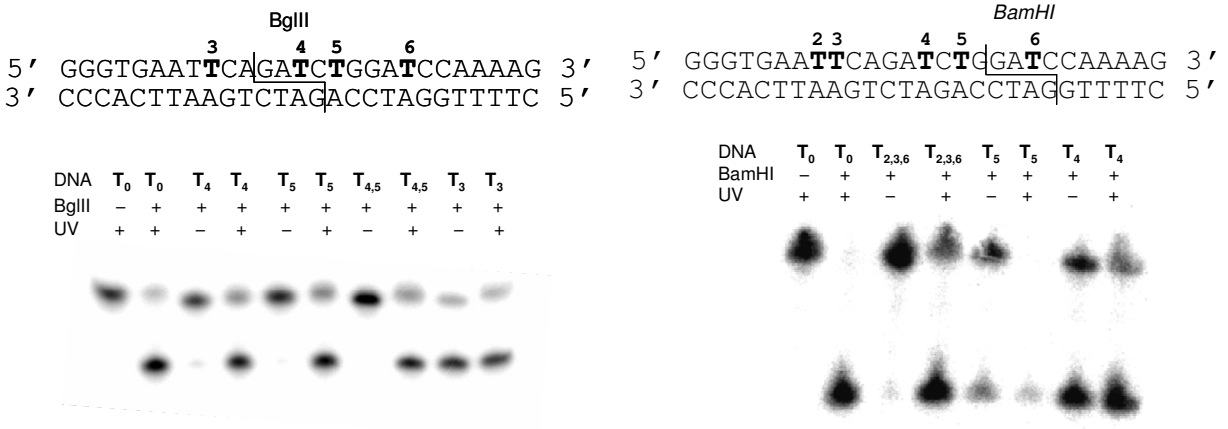
Determination of T_m for Oligonucleotides. Melt curves were measured on a BioRad MyiQ RT-PCR thermocycler by conducting a sequence of 3 heating and cooling cycles (10 µM of both DNA (caged and **T_0**) and complementary DNA with 12.5 µL iQ SYBR Green Supermix to a total volume of 25µL; 30 °C to 80 °C with a 0.5 °C/min ramp). All samples were measured in triplicate and averaged to determine the T_m.



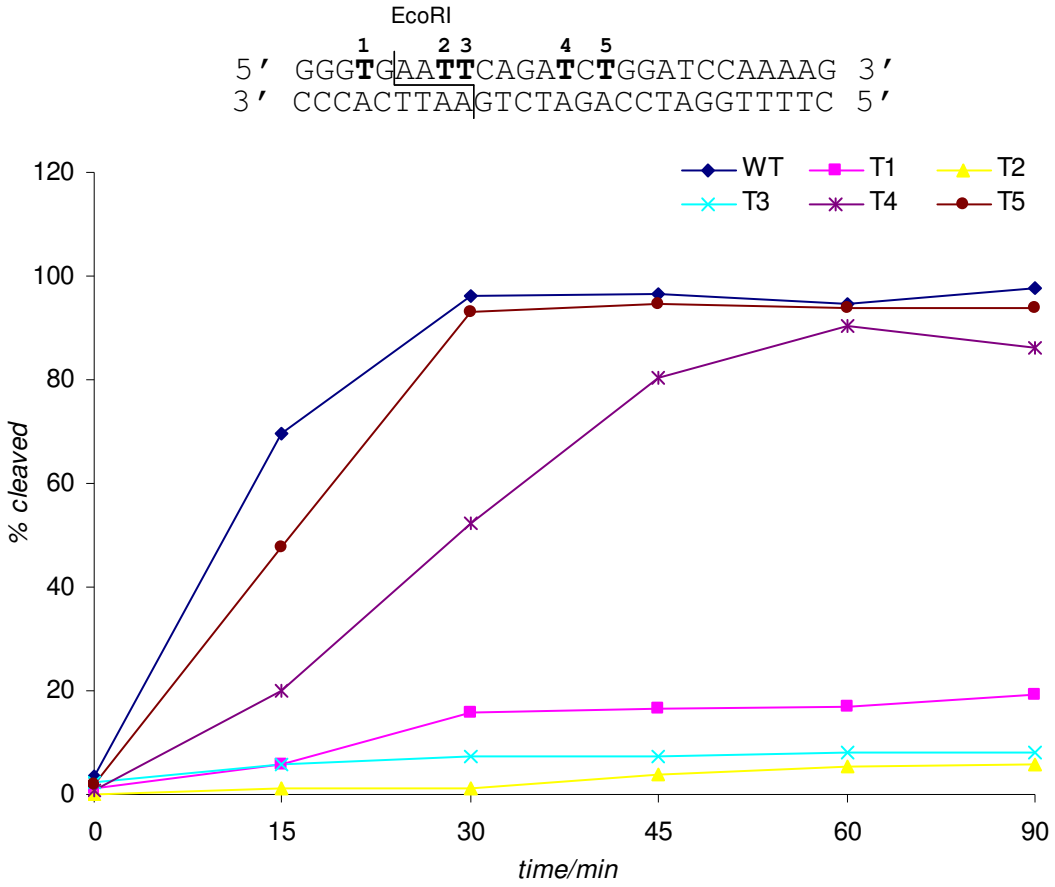
Assessment of the Enzymatic Activity. The γ^{32}P-end labeled $\mathbf{T_0}$ was subjected to a 50 μL enzymatic digest with either EcoRI, BglII, BamHI (as described in the experimental section of the main text), or no restriction endonuclease according to manufacturer's protocols with the appropriate buffer (New England Biolabs). Upon completion, the enzyme was deactivated (70 ^{o}C, 20 min), and digests were analyzed on a 20% denaturing polyacrylamide gel (400V, 40 min). Acrylamide gels were visualized using a Storm phosphorimaging system to assess the ability of each enzyme to digest the non-caged substrate.



Restriction Endonuclease Digests with BglII and BamHI. Similar digests as previously described were performed with BglII and BamHI. Figures 5 and 6 in the main text summarize the results observed in the following gels.

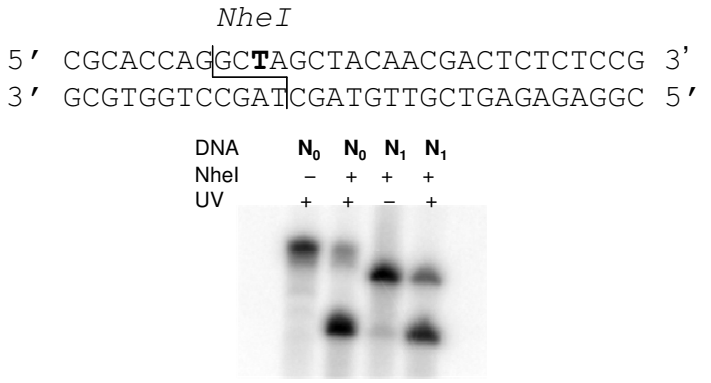


Restriction Enzyme Timecourse. In order to ascertain that the presence of a caging group slows down enzymatic digestion or completely prevents its cleavage, an enzymatic timecourse was performed for *EcoRI* with the caged thymidine at different positions relative to the cleavage site. Based on the timecourse restriction endonuclease activity is completely suppressed, within the error margin of the experiment, if the caged thymidine resides directly within the enzyme recognition/cleavage site. It appears that the effect of the caging group is related to the proximity to the recognition site, as closer caged thymidine residues slow substrate cleavage more dramatically than caging groups further away.

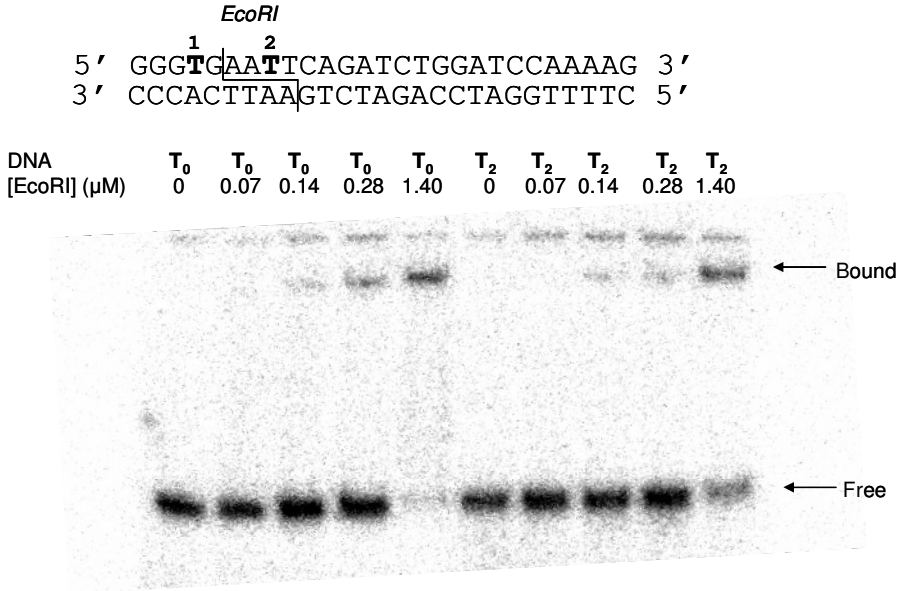


Demonstration of Generality of the Approach via Digestion with Alternative Enzyme. From previous studies we possessed a separate caged substrate containing a caged thymidine within an NheI restriction site. A γ^{32}P-end labeled complementary DNA substrate (10 μL; 1 nmol) was incubated with either $\mathbf{N_0}$ or $\mathbf{N_1}$ (10 μL; 1 nmol) at 90 ^{o}C for 1 min, and then gradually cooled to 4 ^{o}C over 2 hours. The dsDNA construct (2 μL; 0.1 nmol) was then either not irradiated, or irradiated for 5 minutes at 365 nm (25W on a transilluminator), and subjected to a 50 μL enzymatic digest with NheI with the appropriate buffer (New England Biolabs) at 37 ^{o}C for 1 hour . Upon completion, the enzyme was deactivated (70 ^{o}C, 20 min), and digests were analyzed on a 20% denaturing polyacrylamide gel (400V, 40 min). Acrylamide gels were visualized using a Storm phosphorimaging system. Based on the gel data it is apparent that the caging is generally

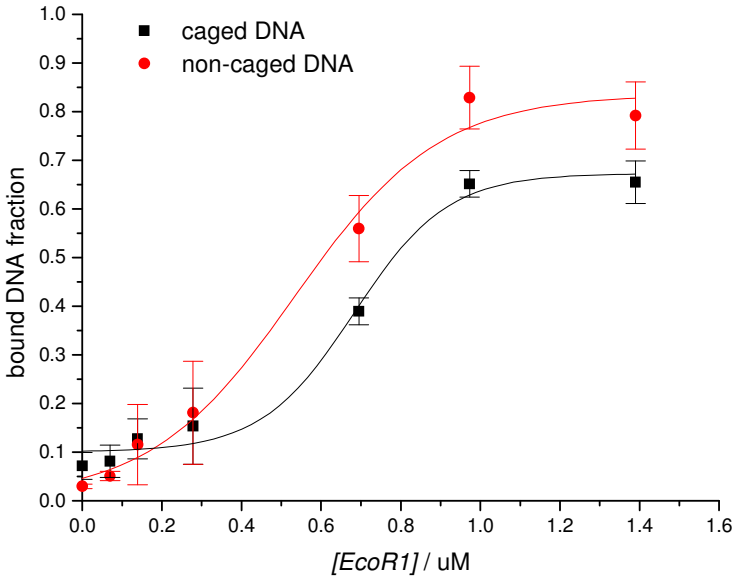
applicable for the photoregulation of restriction endonucleases, as a separate caged substrate was not able to be cleaved by a restriction enzyme unless irradiated with light.



Gel Shift Binding Assay. The γ^{32}P-end labeled complementary strand (10 µL; 1 nmol) was incubated with either **T_0** or **T_2** (10 µL; 1 nmol) at 90 °C for 1 min, and then gradually cooled to 4 °C over 2 hours. Either a 1:10 dilution or a 1:100 dilution of the dsDNA enzyme substrate was made with dH_20, to assess the optimal concentration for detection of a gel shift. The dsDNA construct was then incubated with different concentrations of EcoRI (0, 0.07, 0.14, 0.28, 0.70, 0.98, and 1.40 µM) in binding buffer containing no Mg^{2+} (20 mM Tris, 1 mM EDTA, 5% sucrose, 10 mM DTT, pH 8.0) in a total volume of 40 µL for 1 hour at 25 °C. The sample (5 µL) was then combined with loading buffer (5 µL, 20 mM Tris, 20% glycerol, 1% bromophenol blue, pH 6.8) and analyzed on a 12% native polyacrylamide gel (300V, 20 min). Incubations were repeated in triplicate to accurately determine the binding constants of the enzymes to the substrate. Acrylamide gels were visualized using a Storm phosphorimaging system, and radioactive band intensities were quantified using Image Quant 5.2.

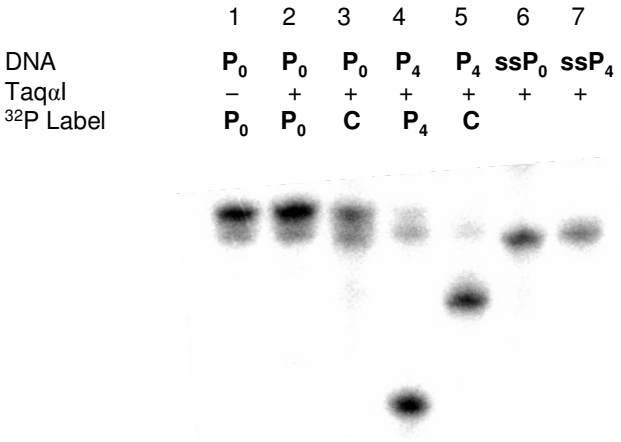


Results of the gel shift assay to determine the binding constants for non-caged and caged *EcoRI* substrates:



Restriction Endonuclease Digests TaqαI. In order to further probe the ability of the enzyme to cleave at 37 °C in the presence of a caging group, all strands (**P_0, P_4,** and **C**) were end labeled with γ^{32}P. The oligonucleotides were either hybridized with their complement (with a single ^{32}P label per dsDNA), or kept single stranded. The DNA was then digested at 37 °C for 2 hours and analyzed a 20% denaturing polyacrylamide gel. In the case of non-caged dsDNA (**P_0** with **C**), neither strand is digested (lanes 2 and 3); however, the caged dsDNA (**P_4** with **C**) resulted in the cleavage of both strands (lanes 4 and 5). The single stranded DNA was not cleaved irrespective of the presence of a caging group (lanes 6 and 7).

	Taqα I	T_m
P_0:	5′ CTGATTTCGACCAGGTTCG 3′	65 °C
C:	3′ GACTAAAGCTGGTCCAAGC 3′	
P_4:	5′ CTGATT**T**CGACCAGGTTCG 3′	55 °C



To ascertain if this caged substrate (**P_4**) was simply prone to degradation by any restriction endonuclease, we subjected it to standard digestion conditions in the presence of a variety of enzymes at 37 ^{o}C. These enzymes are not capable of cleaving the substrate, signifying that only the TaqαI is capable of recognizing and cleaving the caged substrate.