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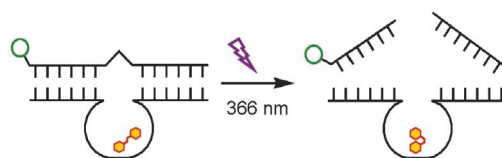
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RNA Cleavage**Reversible Photocontrol of Deoxyribozyme-Catalyzed RNA Cleavage under Multiple-Turnover Conditions**

Lights, camera, action! Photoswitchable nucleoside analogues containing *o*-, *m*-, or *p*-azobenzenes can be inserted in the catalytic core of RNA-cleaving 10–23 deoxyribozymes by replacing a nonconserved residue (see picture). Irradiation of the modified deoxyribozymes at 366 nm enhances RNA cleavage rates up to ninefold, thus achieving the rates observed for the unmodified deoxyribozyme.

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RNA Cleavage

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Reversible Photocontrol of Deoxyribozyme-Catalyzed RNA Cleavage under Multiple-Turn-over Conditions**

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Light is a highly effective and well-established bioorthogonal trigger by which the chemical or biochemical reactivities of nucleic acids can be localized in time and space.^[1] Typically, photoactivation results from the irreversible removal of a masking group from strategic functionalities and has been utilized for the construction of DNA arrays, the study of RNA

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cases $Z \rightarrow E$ photoisomerization was complete following irradiation at 435 nm for 2 min.

Both azobenzene-modified and unmodified (wild-type) deoxyribozymes (wt DRs) catalyzed the site-specific cleavage of a 13-mer oligoribonucleotide substrate labeled at its 3' terminus with a fluoresceinyl moiety (FAM) to yield a labeled hexamer and a 2',3' cyclic phosphate-terminated heptamer. The cleavage reactions were thus resolved, visualized, and quantified in a polyacrylamide gel electrophoresis (PAGE) assay (Figure 1 a). Deoxyribozyme solutions were exposed to light at 366 nm for 10 min or at 435 nm for 2 min, and reactions were initiated by the addition of substrate RNA followed by incubation at 26 °C in the absence of light. To assess the cleavage activity of the more thermally labile irr-*p*-DR, continuous irradiation at 366 nm was performed during the assay.

Under multiple-turnover conditions, irradiated azobenzene-deoxyribozymes maintained essentially wild-type activities; thus, irr-*o*-DR, irr-*m*-DR, and irr-*p*-DR showed cleavage rates of 100, 90, and 50%, respectively (Figure 1 b). In contrast, RNA cleavage rates by dark-adapted (d-a) deoxyribozymes were considerably attenuated. The $k_{\text{irr}}/k_{\text{d-a}}$ ratios in this assay were determined to be 9:1 for *o*-DR and *p*-DR, and 8:1 for *m*-DR (Figure 1 c). Photocontrol of RNA cleavage by deoxyribozyme-azobenzene conjugates was also demonstrated by using an unlabeled RNA substrate and reversed-phase HPLC analysis.^[14] The effects observed in these assays compare well with the results from the PAGE analyses. Thus, the relative rates of substrate cleavage by irr-*o*-DR and irr-*m*-DR are both the same as for the unmodified deoxyribozyme wt DR, and irr-*p*-DR shows 44% of the wild-type activity. Dark-adapted deoxyribozymes give significantly less conversion than the irradiated constructs; $k_{\text{irr}}/k_{\text{d-a}}$ discrimination factors of 6 for *o*-DR and 5 for *m*-DR and *p*-DR constructs were observed.

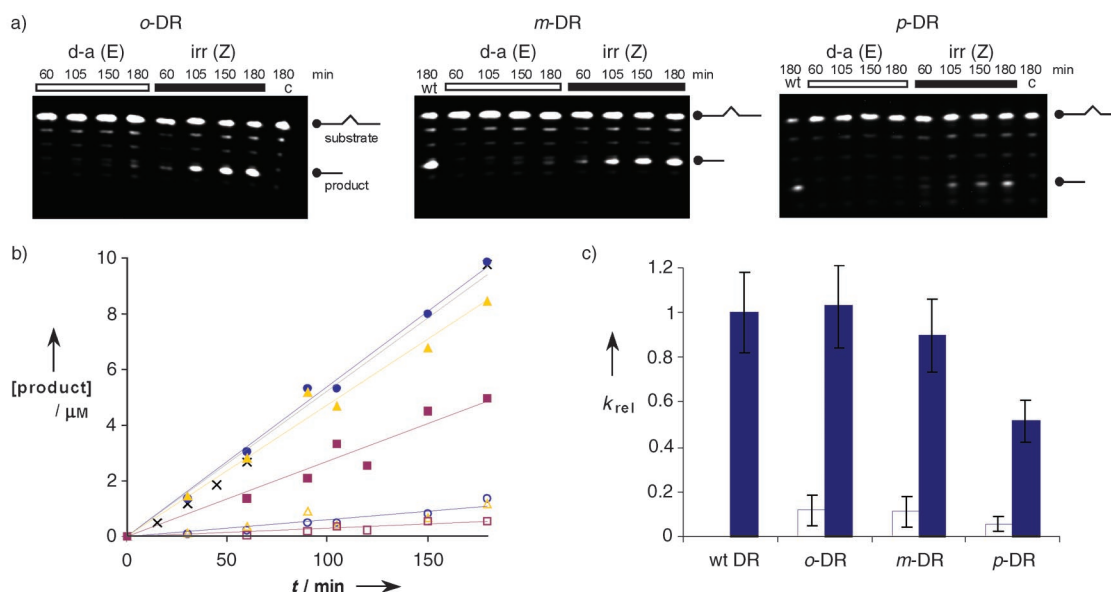


Figure 1. RNA (20 μM) cleavage by 10–23 deoxyribozymes *o*-DR, *m*-DR, *p*-DR, and wt DR under multiple-turnover conditions (10:1 RNA/deoxyribozyme). a) PAGE analysis of 3'-FAM-labeled RNA substrate strands (wt: wt DR RNA cleavage; c: control without DR). b) Quantitative analysis of product formation; key: ○ (d-a)-*o*-DR; ● irr-*o*-DR; △ (d-a)-*m*-DR; ▲ irr-*m*-DR; □ (d-a)-*p*-DR; ■ irr-*p*-DR; × wt DR. c) k_{rel} values for dark-adapted (open bars) and irradiated (filled bars) deoxyribozymes normalized to the unmodified deoxyribozyme reaction.

The effect of photoswitching upon catalysis by the azobenzene-conjugated deoxyribozymes was also demonstrated to be reversible under multiple-turnover conditions with either 5 mol % (*o*-DR or *m*-DR) or 10 mol % (*p*-DR) deoxyribozyme (Figure 2). After initial irradiation of deoxy-

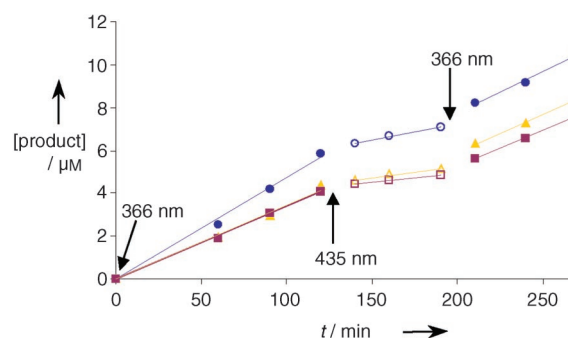


Figure 2. Reversibility of RNA (20 μM) cleavage by 10–23 deoxyribozymes *o*-DR (1 μM), *m*-DR (1 μM), and *p*-DR (2 μM) under multiple-turnover conditions. Key: ○ (d-a)-*o*-DR; ● irr-*o*-DR; △ (d-a)-*m*-DR; ▲ irr-*m*-DR; □ (d-a)-*p*-DR; ■ irr-*p*-DR.

ribozyme solutions at 366 nm the reactions were initiated by addition of substrate, and RNA cleavage rates comparable to those previously described were observed. After 120 min the reactions were irradiated at 435 nm for 2 min to afford the dark-adapted deoxyribozymes which gave rise to significantly retarded reaction rates, although these were higher than those previously observed using purified *E*-DRs. Thus, $k_{\text{irr}}/k_{\text{d-a}}$ discrimination factors of 3.6 for *o*-DR, 3.8 for *m*-DR, and 4.1 for *p*-DR were observed. This difference might be accounted for by perturbation of the $Z \rightarrow E$ photoisomerization process^[4] in the presence of substrate RNA or kinetic

folding traps, which do not respond to this isomerization.^[15] However, the initial cleavage rates were recovered following irradiation at 366 nm (Figure 2).

Large differences in the steric demands and hydrophobic characters of the *E* and *Z* isomers of *para*-azobenzene residues attached to biomolecules are well-described,^[16] but we are unaware of any other report in which the *ortho* isomers give similar activity switching. Preliminary NMR spectroscopic investigations indicate that both *E* and *Z* isomers of *p*-1 reside in the *C2'-endo* furanoside pucker typical of 2'-amido deoxyribonucleoside analogues and their unmodified congeners, and so the active conformation of the catalytic core containing the photoswitch may be modulated in some other fashion.

Our demonstration of photomodulated deoxyribozyme-catalyzed RNA cleavage under multiple-turnover conditions is of particular interest as the irradiated “on” state maintains wild-type cleavage rates. The novel analogues described herein enable incorporation of azobenzene moieties with readily accessible nucleoside derivatives, which have the potential to maintain essential base contacts and the biological activity of nucleic acids. We envisage that the ability to reversibly modulate the catalytic RNA cleavage rates of the 10–23 deoxyribozyme by light will add a useful tool to the repertoire of regulatory biocatalysts. We are currently working toward the development of light-programmable conformational switches within DNA and RNA,^[17] and their application to the spatiotemporal control of gene expression and array-based computation.^[18]

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- [1] J. H. Kaplan, B. Forbush, J. F. Hoffman, *Biochemistry* **1978**, *17*, 1929–1935.
- [2] a) A. C. Pease, D. Solas, E. J. Sullivan, M. T. Cronin, C. P. Holmes, S. P. A. Fodor, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5022–5026; b) S. G. Chaulk, A. M. MacMillan, *Nucleic Acids Res.* **1998**, *26*, 3173–3178; c) C. Höbartner, S. K. Silverman, *Angew. Chem.* **2005**, *117*, 7471–7475; *Angew. Chem. Int. Ed.* **2005**, *44*, 7305–7309; d) P. Wenter, B. Fürting, A. Hainard, H. Schwalbe, S. Pitsch, *Angew. Chem.* **2005**, *117*, 2656–2659; *Angew. Chem. Int. Ed.* **2005**, *44*, 2600–2603; e) R. Ting, L. Lermer, D. M. Perrin, *J. Am. Chem. Soc.* **2004**, *126*, 12720–12721; f) A. Heckel, G. Mayer, *J. Am. Chem. Soc.* **2005**, *127*, 822–823; g) S. Shah, S. Rangarajan, S. H. Friedman, *Angew. Chem.* **2005**, *117*, 1352–1356; *Angew. Chem. Int. Ed.* **2005**, *44*, 1328–1332; h) H. Ando, T. Furuta, R. Y. Tsien, H. Okamoto, *Nat. Genet.* **2001**, *28*, 317–325; i) W. T. Monroe, M. M. McQuain, M. S. Chang, J. S. Alexander, F. R. Haselton, *J. Biol. Chem.* **1999**, *274*, 20895–20900.
- [3] a) H. Asanuma, T. Ito, T. Yoshida, X. G. Liang, M. Komiyama, *Angew. Chem.* **1999**, *111*, 2547–2549; *Angew. Chem. Int. Ed.* **1999**, *38*, 2393–2395; b) A. Yamazawa, X. G. Liang, H. Asanuma, M. Komiyama, *Angew. Chem.* **2000**, *112*, 2446–2447; *Angew. Chem. Int. Ed.* **2000**, *39*, 2356–2357; c) H. Asanuma, D. Tamaru, A. Yamazawa, M. Z. Liu, M. Komiyama, *ChemBioChem* **2002**, *3*, 786–789.
- [4] G. A. Woolley, *Acc. Chem. Res.* **2005**, *38*, 486–493.
- [5] I. Willner, S. Rubin, A. Riklin, *J. Am. Chem. Soc.* **1991**, *113*, 3321–3325.
- [6] a) M. Banghart, K. Borges, E. Isacoff, D. Trauner, R. H. Kramer, *Nat. Neurosci.* **2004**, *7*, 1381–1386; b) M. Volgraf, P. Gorostiza, R. Numano, R. H. Kramer, E. Y. Isacoff, D. Trauner, *Nat. Chem. Biol.* **2006**, *2*, 47–52.
- [7] L. Guerrero, O. S. Smart, G. A. Woolley, R. K. Allemann, *J. Am. Chem. Soc.* **2005**, *127*, 15624–15629.
- [8] Y. Liu, D. Sen, *J. Mol. Biol.* **2004**, *341*, 887–892.
- [9] a) S. W. Santoro, G. F. Joyce, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4262–4266; b) S. W. Santoro, G. F. Joyce, *Biochemistry* **1998**, *37*, 13330–13342.
- [10] a) H. Suenaga, R. Liu, Y. Shiramasa, T. Kanagawa, *Appl. Environ. Microbiol.* **2005**, *71*, 4879–4884; b) I. H. Chang, J. J. Tulock, J. W. Liu, W. S. Kim, D. M. Cannon, Y. Lu, P. W. Bohn, J. V. Sweedler, D. M. Crokek, *Environ. Sci. Technol.* **2005**, *39*, 3756–3761; c) D. Y. Wang, D. Sen, *J. Mol. Biol.* **2001**, *310*, 723–734; d) S. H. Pun, F. Tack, N. C. Bellocq, J. J. Cheng, B. H. Grubbs, G. S. Jensen, M. E. Davis, M. Brewster, M. Janicot, B. Janssens, W. Floren, A. Bakker, *Cancer Biol. Ther.* **2004**, *3*, 641–650.
- [11] a) Z. Zaborowska, S. Schubert, J. Kurreck, V. A. Erdmann, *FEBS Lett.* **2005**, *579*, 554–558; b) Z. Zaborowska, J. P. Furst, V. A. Erdmann, J. Kurreck, *J. Biol. Chem.* **2002**, *277*, 40617–40622.
- [12] a) C. R. Dass, E. G. Saravolac, Y. Li, L. Q. Sun, *Antisense Nucleic Acid Drug Dev.* **2002**, *12*, 289–299; b) M. J. Cairns, T. M. Hopkins, C. Witherington, L. Wang, L. Q. Sun, *Nat. Biotechnol.* **1999**, *17*, 480–486; c) S. K. Silverman, *Nucleic Acids Res.* **2005**, *33*, 6151–6163.
- [13] D. P. C. McGee, A. Vaughnsettle, C. Vargeese, Y. S. Zhai, *J. Org. Chem.* **1996**, *61*, 781–785.
- [14] See the Supporting Information for details.
- [15] a) K. Nakayama, M. Endo, T. Majima, *Chem. Commun.* **2004**, 2386–2387; b) A. M. Caamano, M. E. Vazquez, J. Martinez-Costas, L. Castedo, J. L. Mascarenas, *Angew. Chem.* **2000**, *112*, 3234–3237; *Angew. Chem. Int. Ed.* **2000**, *39*, 3104–3107.
- [16] C. Dugave, L. Demange, *Chem. Rev.* **2003**, *103*, 2475–2532.
- [17] R. Micura, *Angew. Chem.* **2006**, *118*, 32–34; *Angew. Chem. Int. Ed.* **2006**, *45*, 30–31.
- [18] M. N. Stojanovic, D. Stefanovic, *Nat. Biotechnol.* **2003**, *21*, 1069–1074.