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Optical Control of Enzyme Enantioselectivity in Solid Phase

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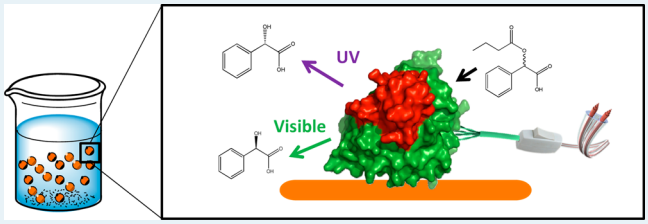
Supporting Information

ABSTRACT: A lipase was immobilized on transparent agarose microspheres and genetically engineered to specifically anchor photochromic molecules into its catalytic site. Several combinations of azobenzene and spiropyran groups were conjugated to cysteines introduced at different positions near the active center. Light modulated the catalytic properties of the resulting solid bioconjugates, and such modulation depended on both the nature of the photochromic compound and the anchoring position. Covalent anchoring of azobenzene derivatives to the residue 295 of the lipase 2 from *Bacillus thermocathenolatus* triggered lipase preference for the *S* isomer under UV light, whereas visible light promoted preference for the *R* isomer. Molecular dynamics simulations indicate that conjugating photochromic compounds into the catalytic cavity allows manipulating the steric hindrance and binding energy of the substrates, leading to an enantioselective molecular fit in certain cases. Using this approach, we report for the first time the control of enzyme properties using light in the solid phase.



KEYWORDS: *lipase, azo compounds, photochromism, chemical modification, immobilization*

INTRODUCTION

Isolated enzymes can catalyze organic transformations at both high yield and high enantioselectivity under mild conditions. However, in situ modulation of catalytic properties has proven a great challenge, as indicated by the lack of methods to regulate enzyme enantioselectivity during in vitro biotransformations. The dynamic control of enzyme selectivity for in vitro reactions schemes is thus an unmet need, especially in the context of cascade reactions catalyzed by multienzyme systems, in which biocatalysts must be switched on and off in situ according to system requirements. Optical control[1] offers the possibility to remotely manipulate enzyme activity using spatiotemporally designated patterns of illumination.[2] Moreover, the immobilization of these engineered biocatalysts would enable their reuse as well as their incorporation into nanodevices.[3] Here, we present a rational approach to conjugate photochromic compounds to an immobilized enzyme in a site-directed manner and demonstrate for the first time the regulation of its enantioselectivity with light.

Lipases are serin−threonin hydrolases that naturally catalyze the hydrolysis of lipids and are widely applied in chemical processes from research laboratories to industrial plants.[4] Most lipases present a hydrophobic active site shielded by an amphiphilic domain (named as "lid") that triggers the catalytic mechanism in the presence of hydrophobic substrates.[5] This class of enzymes is the paradigm of enantioselective biocatalysis, and their hydrolytic rate and enantiomeric excess can be enhanced by adjusting the position of the substrate into the hydrophobic cavity.[6] A plethora of methodologies to alter the lipase catalytic mechanism have been devised, including enzyme engineering, reaction media engineering, immobilization, and chemical modification.[6−8] We have recently reported the alteration of both activity and selectivity of lipase 2 from *Bacillus thermocathenolatus* (BTL2) using site-directed chemical modification in the solid phase.[9] Building upon this methodology, we pursued photocontrol of BTL2 catalytic properties by tethering a photochromic group inside the enzyme active center.

RESULTS AND DISCUSSION

Site-Directed Chemical Modification of BTL2 in Solid Phase. We chemically modified BTL2 with different photo-

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chromic molecules. As a result of the reversible transformation of the photochromic group anchored to the protein scaffold, we expected the active center conformation to change under different illumination conditions (Figure 1). To test whether

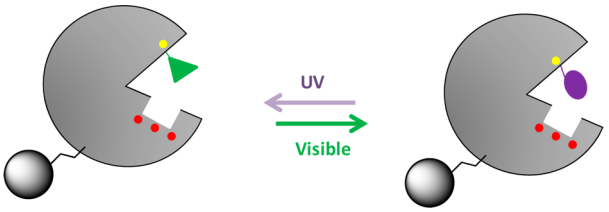

Figure 1. Site-directed chemical modification of BTL2 with phoswitchable molecules. Reversible conformational changes of semisynthetic binding pocket induced by light. BTL2 immobilized on agarose beads activated with cyanogen bromide groups has been site-directed-modified with photoswitchable molecules. Since the photosensitive molecules have been closely anchored to the catalytic residues, enzyme properties can be modulated.

those changes availed in altering the enzyme activity, we evaluated several positions located at the substrate binding pocket and two compounds displaying different photochromic processes (Figure 2A,B). In particular, an iodoacetate–spiropyran (**1**, Figure 2A) and an azobenzene–methylthiosulfonate (**2**, Figure 2B) were specifically conjugated to a unique cysteine at positions 17, 187, 245, 295, and 320 of BTL2. These positions were rationally chosen because they are important either for the substrate binding (17, 245 and 320) or for the lid aperture during the catalysis (187 and 295) (Supporting Information (SI) Figure S1 and Table S1).[5] The unique cysteine at the specific position was introduced by site-directed mutagenesis using a cysteine-less BTL2 variant that was created in previous work[9] to avoid unspecific chemical modification.

Both catalytic rate and enantioselectivity were assayed toward a survey of esters, under different light conditions (see the SI for details on the synthesis, mutagenesis, purification and site-selective chemical modification in solid phase of the enzymes). The conjugation took place in the solid phase by incubating the immobilized BTL2 in a photochromic compound solution. The yield of the chemical modification was evaluated by thiol titration. As an example, 94 ± 9 (%) of the BTL2 active centers were modified with molecule **1** at position 245. The conjugation procedure was simplified by solid-phase preparation because excess reagents and solvents could be easily removed by vacuum filtration. The site-directed chemical modification was confirmed by mass spectrometry (SI Figure S2)

Spectroscopic Characterization of the Photosensitive Biocatalysts. The photochromic biocatalysts were spectroscopically characterized under different illumination conditions. Ultraviolet (UV) light transforms the apolar spiropyran **1** into the planar, polar merocyanine form (Figure 2A) and causes the isomerization of azo derivative **2** from the extended trans configuration to the bent cis form (Figure 2B). In both cases, the initial state is reversibly recovered under visible illumination or after thermal relaxation in darkness (Figure 2C,D). It is noteworthy that photochromic molecules tethered to immobilized BTL2 (**1b** and **2b**) presented absorbance spectra similar to those of the molecules in solution. UV light increased the absorbance of compound **1** at 550 nm, as reported[10] (Figure 2C, inset) and decreased the absorbance compound **2** at 350 nm, as described for similar azobenzenes[11] (Figure 2D, inset).

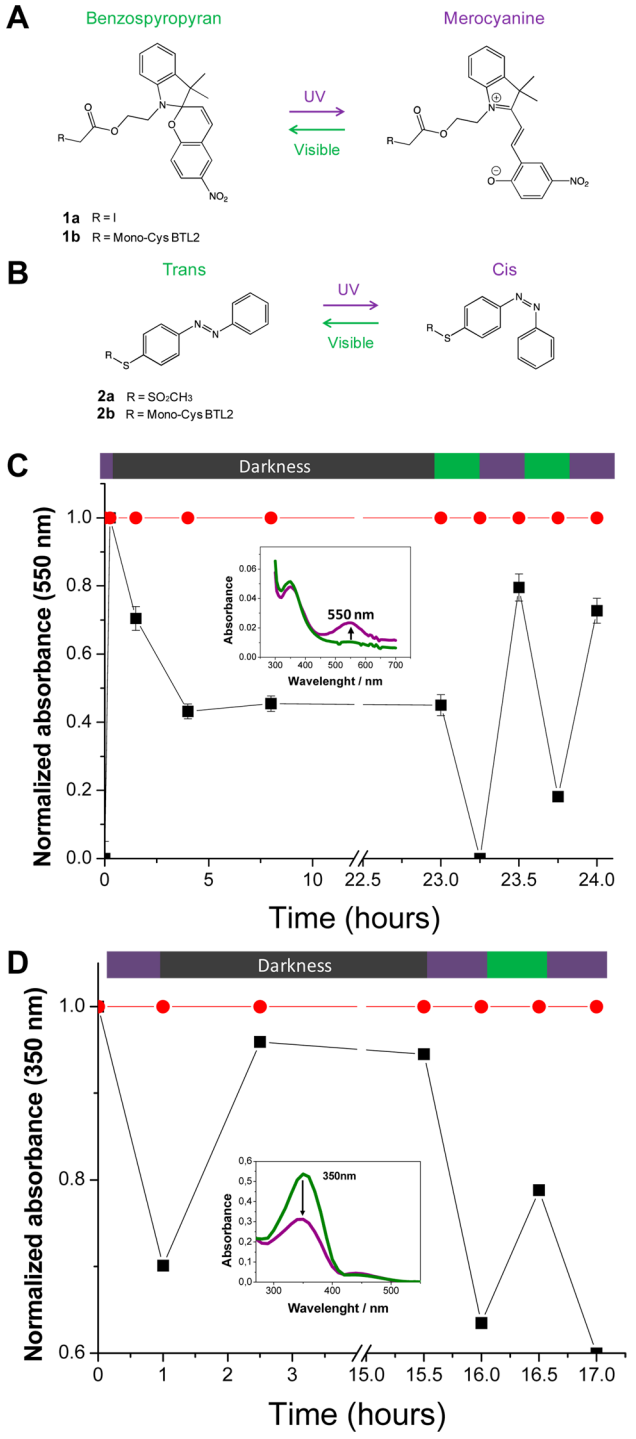

Figure 2. Photochromic properties of different immobilized bioconjugates. Photoswitchable equilibrium undergone by spiropyran (A) and azobenzene (B) molecules. The spiropyran compound is activated by an iodoacetate reactive group (**1a**) to specifically react to cysteines on the protein structure, resulting in covalent and irreversibly anchored (**1b**) bioconjugates. In the same way, the azobenzene compound was activated with methylthiosulfonate groups (MTS) (**2a**) that selectively react to cysteine, as well, but form covalent and reversible bioconjugates (**2b**). Spectroscopic characterization of immobilized BTL2 bioconjugates modified with either spiropyran (C) or azobenzene (D) molecules (see Supporting Information). Inset figure depicts the absorbance spectrum of solid bioconjugates illuminated with either visible (green line) or UV (violet line) light. The arrows point out the effect of the light switch on the absorbance maximum of the different molecules. The main graph represents the

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Figure 2. continued

evolution of active site states under different light conditions. Red circles correspond to wild type and, consequently, nonmodified BTL2; black squares, to BTL2-modified at position 245. After illuminating solid photochromic bioconjugates with UV lights, the relaxation of the excited compound was monitored along the time in darkness. Afterward, the solid preparations were subjected to light cycles to study the photoisomerization of the anchored molecules into the active site environment.

However, the half-life of thermal relaxation and the stationary state achieved in the dark by both photochromic molecules were substantially altered by coupling to the protein.[12] For example, after UV illumination, the soluble **1a** relaxation rate under darkness was 20% faster than the same molecule anchored to position 245 in the BTL2 active site (**1b**). In fact, 80% of soluble **1a** was relaxed after 24 h in darkness, but only 60% relaxation was observed after 24 h for **1a** conjugated to the protein scaffold (**1b**). Moreover, **1a** and **1b** preparations only partially relaxed in the dark and required visible illumination to completely convert them back to the spiropyran form (SI Figure S3A, Figure 2C). Therefore, the alteration of the "reversible photo-equilibrium" suggests that the protein environment could stabilize the polar merocyanine, as previously reported.[13]

Effect of Light on Immobilized BTL2 Catalytic Properties. Catalytic reactions with several conjugated BTL2 variants were carried out under different illumination conditions, and the activities were measured using colorimetric assays (see the SI). Figure 3A and B shows the hydrolytic activity of different immobilized bioconjugates under UV and visible light. In general, conjugation of photochromic molecules led to a reduction in lipase activity, but several variants displayed a catalytic rate that was moderately light-dependent, thus validating the strategy to photocontrol an enzyme with a shielded active site. As an example, BTL2 conjugated with **1** at positions 187 and 245 (BTL2−187C-**1** and BTL2−245C-**1**) and with **2** at position 245 (BTL2−245C-**2**) presented a relative 1.5-fold higher activity under UV light than under visible light (Figure 3A and B). These results demonstrate that the anchoring site of the photochromic group in the lipase is critical to control its hydrolytic activity with light and constitute the first demonstration of enzyme photoregulation in the solid state using porous materials as carriers. The choice of agarose beads as carriers was key because its transparency allows light passing through the pores and illuminating the enzymes attached to the solid structure, thereby activating the photochromic molecules anchored to the protein.

These novel results prompted us to examine enzyme enantioselectivity, arguably one of the most outstanding properties of lipases.[6] To that end, we tested racemic mixtures as substrates and measured the chirality of products yielded by different BTL2 bioconjugates under UV and visible illumination. The enantioselectivity of modified BTL2 variants toward two different racemic esters, 2-butyryloxy-2-phenylacetic acid (***rac*-7**) and 1-phenyl-ethyl acetate (***rac*-8**), is shown in Figure 3C and D, respectively. In the first case, site-directed modification of BTL2 with compound **1** tended to preferentially hydrolyze the *S* enantiomer, inverting the natural preference by the *R* enantiomer displayed by the native enzyme. In contrast, modification with compound **2** enhanced the enantiomeric excess values of the native enzyme (see

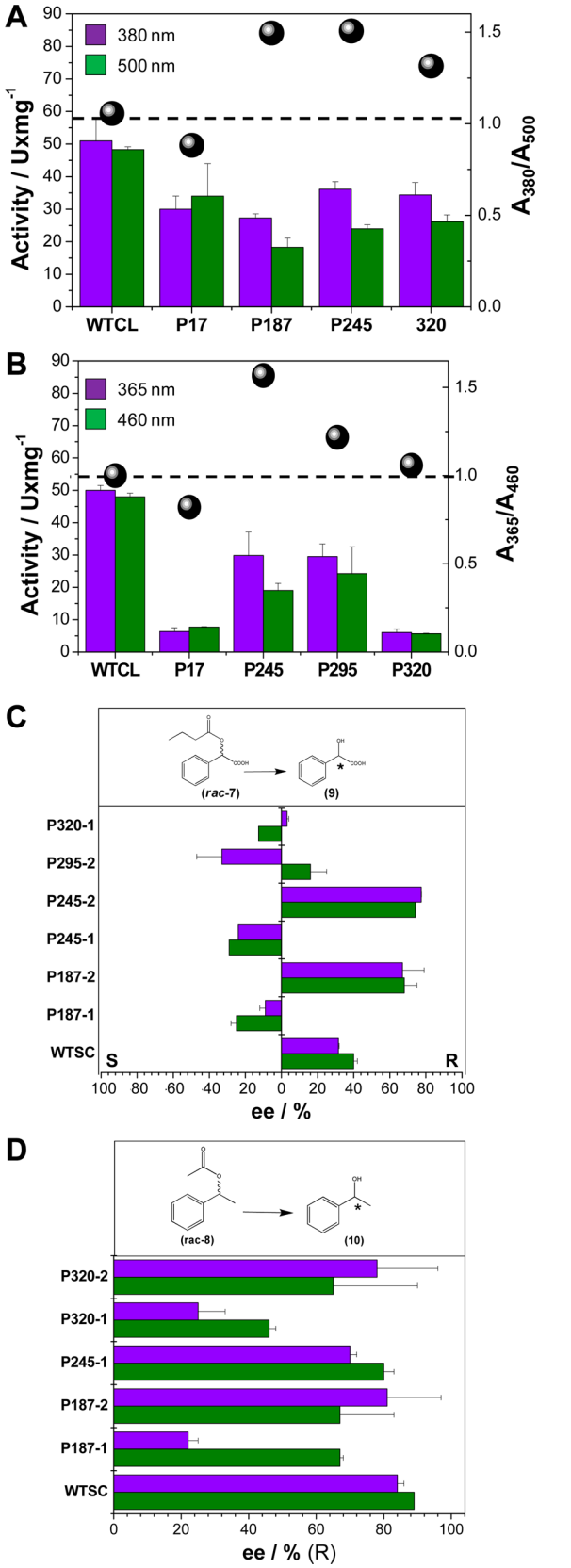

Figure 3. Analysis of the enzyme properties of photoswitchable lipase under different illumination conditions. Esterase activity of different immobilized variants of BTL2 modified with either **1a** (A) or **2a** (B) compounds under light at different wavelengths. Hydrolytic activity of the different chemically modified BTL2 variants were measured under either visible light (green bars) (500 and 460 nm for **1b** and **2b**

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Figure 3. continued

bioconjugates respectively) or UV light (violet bar) (380 and 365 nm for **1b** and **2b** bioconjugates, respectively). The spherical points show the photoisomerization ratio defined as the coefficient between the specific enzyme activity under UV and visible lights. Values close to 1 mean a non photoswitchable lipase, and ratios higher or lower than 1 mean photoswitchable lipases. Selective hydrolysis of 2-butyryloxy-2-phenylacetic acid (*rac*-**7**) (C) and 1-phenyl-ethyl acetate (**rac-8**) (D) to their corresponding chiral alcohols 3-phenyl-2-hydroxypropionic acid (**9**) and 1-phenyl ethanol (**10**) catalyzed by different BTL2 bioconjugates modified with either **1a** or **2a** compounds. Effect of light on the lipase selectivity was determined by measuring the enantiomeric excess (ee; %) of the different bioconjugates modified at different positions under either visible (green bars) or UV (violet bars) light. For bioconjugates **1b**, visible and UV wavelengths were 500 and 380 nm; for bioconjugates **2b**, they were 460 and 365 nm for visible and UV light, respectively.

complete results in SI Tables S2 and S3). Photocontrol of the enantioselectivity depended both on the conjugation site and on the nature of the photochromic molecule, unlike activity that depended on only the conjugation position. In this regard, the enantioselectivity for the hydrolysis of *rac*-**7** could be inverted with light by using BTL2−320C-**1** (the *S* enantiomer is favored under visible light) and BTL2−295C-**2** (the *S* enantiomer is favored under UV light) (Figures 3C and SI S4). On the other hand, enantioselectivity changes were also observed in the hydrolysis of *rac*-**8** catalyzed by BTL2−187C-**1** and BTL2−320C-**1** (the *R* enantiomer is favored under visible light) (Figure 3D). In particular, the enantioselectivity of BTL2−187C-**1** toward *rac*-**8** under visible light was 3 times higher than under UV light and approached that of the native enzyme.

Position 320 located at the binding pocket and the contact region between the lid domain and the active site are "hot-spots" to anchor photochromic molecules that effectively modulate the enzyme enantioselectivity by the action of the light. Similarly, chemical modification of position 320 of BTL2 with alkanes modified both the activity and the enantioselectivity of the immobilized BTL2.[9]

In principle, this approach can be expanded to other enantioselective enzymes. In fact, the activity of several enzymes can be modulated by light regardless both the morphology of their active sites and the catalytic mechanisms (endonucleases, carbonic anhydrase, etc.).[14−16] Therefore, these light-driven changes in enzyme enantioselectivity constitute a breakthrough in the regulation of biocatalysis for in vitro applications that complements engineered[17] and artificial[18−20] enzyme strategies to provide reversibility and remote control and largely outperforms previous attempts using additives in the reaction media.[9] Moreover, this work proves for the first time that light can also modulate enzyme properties in the solid phase as well as it does in solution. The resulting photochromic immobilized biocatalysts might be used in a fix-bed reactor for a continuous process that is able to respond to external signals, such as the light. This opens new opportunities for the regulation of biocatalytic systems formed by isolated enzymes.

Effect of Light on Catalytic Versatility of the Photochromic Biocatalysts. Since photoisomerization is expected to produce subtle changes in the catalytic site, we evaluated the photocontrol of the catalytic versatility of these novel biocatalysts. For that purpose, we performed a kinetically controlled transesterification reaction using glycerol (**3**) and methyl butyrate (**4**) as substrates (Figure 4). It has been

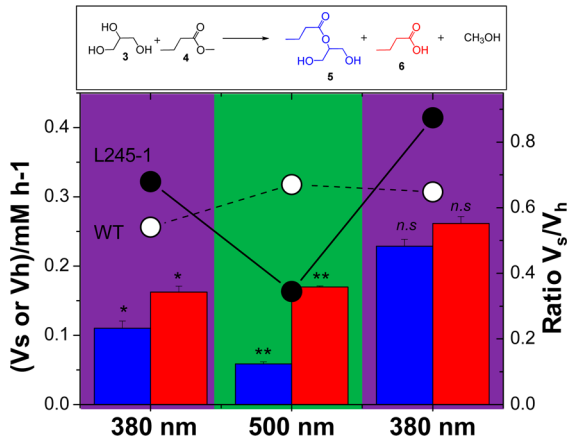

Figure 4. Kinetically controlled synthesis of 2-*O*-butyryl-glycerol (**5**) catalyzed by BTL2 modified with **1a** at position 245 . The synthesis of **5** was carried using glycerol (**3**) and methyl butyrate (**4**) as substrates. In this reaction, the enzyme can also catalyze the hydrolytic reaction of the substrate **4**, yielding butyric acid (**6**) and methanol as the products. The light was switched every 30 min during the reaction, and the substrates and products were analyzed by HPLC to study the simultaneous hydrolytic and synthetic reactions of the mentioned bioconjugate. Synthetic (blue bars) and hydrolytic (red bars) rates were determined under different light conditions. Error bars show the standard deviation of two independent measurements subjected to *t* test analysis, where (*)$p < 0.05$, (**)$p < 0.01$ are the significant differences between hydrolytic and synthetic rates and n.s. means no significant differences between those rates. The black circles and solid black line represent the ratio between the synthetic and the hydrolytic activity of the photoswitchable bioconjugate, and the white circles and dashed black line show the same ratio for the unmodified wild type enzyme.

reported that BTL2 catalyzes the transesterification reaction, yielding the corresponding glyceryl butyrate (**5**). However, the enzyme can also hydrolyze **4**, yielding butyric acid (**6**), undesirably lowering the synthetic product yield.[21] This reaction is highly interesting from a mechanistic point of view because it reveals that the lipase can act as synthetase and hydrolase in the same reaction. The ratio between synthesis and hydrolysis depends on the catalytic properties of the enzyme, and thus, it could be, in principle, regulated by light. To test this possibility, we carried out the transesterification reaction using immobilized BTL2−245-**1** and different light cycles. Figure 4 shows how BTL2 properties were altered by light while the unmodified native lipase was insensitive to illumination. Although UV light promotes an active site configuration that can hydrolyze and synthesize at similar rates, visible light drives an enzyme that hydrolyzes 3 times better than it synthesizes. In the light of the statistical analysis of these results, differences between synthetic and hydrolytic ratios were much more significant under visible light than under UV light; therefore, the synthesis/hydrolysis ratio can be dynamically controlled by light, enabling the temporal control of catalytic performance of BTL2 in mechanistic terms.

In Silico Studies of Photosensitive Bioconjugates. Binding Mode of Substrate to the Active Site under Different Light Conditions. Because the success of this approach was built by rational design but also by testing empirically several attachment positions and reaction con-

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ditions, we turned to computer simulations of the best photocontrolled lipase variants to identify the principles that could help in the optimization of further design. In particular, we asked whether the observed enantiomeric excesses for the different bioconjugates can be explained by the influence of photoisomerization on the spatial availability within the active site by altering the substrate-binding properties (energy and conformation) or by conformational changes in the enzyme structure. To this aim, molecular dynamics (MD) simulations were performed on the lipase modified with either spiropyran or azobenzene molecules (see Online Methods), followed by separate docking calculations with the *R* and *S* enantiomers of the 2-butyryloxy-2-phenylacetic acid (***rac*-7**) substrate. MD simulations have successfully been used to model conformational motions in lipases,[22] including substrate binding.[23] The current MD simulations show that bioconjugation with **1** and **2** does not perturb the protein folding, and the substrate binding pocket does not change significantly. Because the reactions were performed under nonsaturating conditions (1 mM), binding energy differences should reflect differences in reactivity. Analysis of the most populated substrate docking poses (among the ones well oriented for catalysis) (Figure 5)

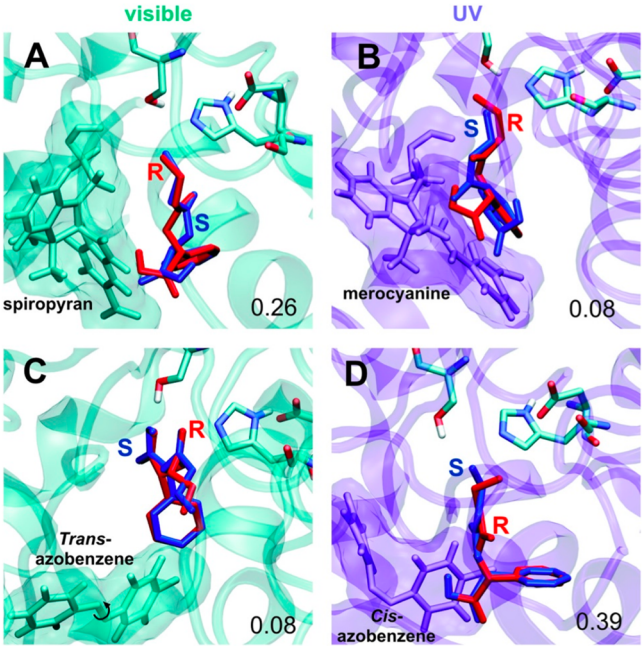

Figure 5. Most populated docking modes of (*R*,*S*)-2-butyryloxy-2-phenylacetic acid (***rac*-7**) in the active site of the bioconjugates BTL2−320C-1(top) and BTL2−295C-2 (bottom) under visible (left) and UV (right) light. The differences in binding energies between the *R* and *S* enantiomers are indicated on the figures (positive values indicate binding that favors the *S* enantiomer).

shows that the *R*/*S* enantiomers have a similar binding energy for the bioconjugates showing little enantiomeric excess (BTL2−320C-**1** in the merocyanine state and BTL2−295C-**2** in the trans configuration, Figure 3C). In contrast, the two enantiomers are well separated in terms of binding energy for the bioconjugates for which the highest enantiomeric excess was measured (BTL2−320C-**1** in the spiropyran state and BTL2−295C-**2** in the cis configuration, Figure 3C). Furthermore, the *S* enantiomer is clearly favored with respect to the *R* enantiomer not only in terms of energy but also in terms of structure (closeness of the substrate to the catalytic residues), with the effect being more pronounced for BTL2−295C-**2** under UV-light and in the cis conformation (Figure 5 and SI Table S4). Therefore, the simulations support that the highest enantiomeric excesses are experimentally observed for these two bioconjugates: BTL2−320C-**1**(top) and BTL2−295C-**2** (bottom) under visible (left) and UV (right) light. The differences in binding energies between the *R* and *S* enantiomers are indicated on the figures (positive values indicate binding that favors the *S* enantiomer).

Several strategies have been devised to control the catalytic rate of enzymes with light,[1] including unspecific conjugation of photochromic compounds,[13] diffusible photochromic ligands,[24] and site-specific incorporation of photochromic unnatural aminoacids.[15] However, photocontrol of chirality has remained elusive, despite the wide biochemical and biotechnological importance of enantioselective enzymes and knowledge of their structure and catalytic mechanisms.

Here, we have presented a rational approach to nanoengineer immobilized enzymes by site-directed chemical modification with photochromic compounds. We have demonstrated how such chemical modifications enable optical regulation of BTL2 catalytic properties in different reaction schemes. The strategy of conjugating photochromic compounds in the binding pocket has allowed manipulation of the steric hindrance of the cavity and achievement of an enantioselective molecular fit in certain cases. Structural modeling and simulations provide a good basis to understand the experimental results, pinpointing the atomic-scale structural elements that are key to photoregulate BTL2 activity and selectivity. In particular, light acts as an external stimulus that influences the active site spatial availability rather than promoting a conformational change in the protein tertiary structure (SI Figure S5).

In this way, light remotely alters the substrate binding because the binding pocket is differently but reversibly shaped by a photocontrolled molecular adaptor. The combination of experiments and simulations suggests that the approach could be extended to other structurally well-characterized enzymes or multienzymatic complexes, providing reversible and non-invasive modulation of different biocatalytic properties in-pot. This novel form of enzymatic photoregulation might be useful to externally control a branching point in cascade enzymatic reactions. For example, in one-pot multienzymatic complexes, including photochromic lipase, light would act as an external stimulus, allowing directing of the chemical signals involved in cascade chemical transformations, that is, selecting the products to be fed as substrates into subsequent enzymatic steps. This function is analogous to that of demultiplexer devices in electronic circuits (SI Figure S6) and has been recently demonstrated in organic molecular devices.[25] The enzyme with photoswitchable enantioselectivity described here thus constitutes the first demonstration of a protein-based demux.[26]

■ CONCLUSION

In summary, we have presented a method to control the catalytic properties of a lipase with light that is based on the site-directed chemical conjugation of photochromic molecules into the catalytic cavity. In particular, we have demonstrated for the first time the control of enzyme enantioselectivity with light by using chemically modified heterogeneous biocatalysts. Molecular dynamics simulations indicated that conjugating photochromic compounds in the active site allows manipulation of the steric hindrance and binding energy of the substrates, leading to an enantioselective molecular fit in certain

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cases. The experimental results and simulations suggest that this approach can be extended to other enzymes of fundamental and biotechnological relevance. Moreover, aided by these insights, we are in a good position to design new positions for the photochromic compounds to anchor so as to enhance the optical response of the immobilized bioconjugates. We can also add new chemical groups to the photochromic compounds to potentiate their supramolecular interactions with the protein scaffold. Therefore, both the position and the nature of the photochromic compounds may enhance the effect of the light on the enzyme properties.

■ ASSOCIATED CONTENT

Supporting Information

Experimetnal details and raw data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Volgraf, M.; Banghart, M.; Trauner, D. *Switchable Proteins and Channels*, 2nd ed.; Wiley-VCH Verlag: Weinheim, 2011; Vol. *2*; Chapter 15; pp 563−593.
(2) Gorostiza, P.; Isacoff, E. Y. *Science* **2008**, *322* (5900), 395−9.
(3) Bolivar, J. M.; Wiesbauer, J.; Nidetzky, B. *Trends Biotechnol.* **2011**, *29* (7), 333−342.
(4) Gröger, H.; Asano, Y.; Bornscheuer, U. T.; Ogawa, J. *Chem.—Asian. J.* **2012**, *7* (6), 1138−1153.
(5) Carrasco-Lopez, C.; Godoy, C.; de Las Rivas, B.; Fernandez-Lorente, G.; Palomo, J. M.; Guisan, J. M.; Fernandez-Lafuente, R.; Martinez-Ripoll, M.; Hermoso, J. A. *J. Biol. Chem.* **2009**, *284* (7), 4365−72.
(6) Berglund, P. *Biomol. Eng.* **2001**, *18* (1), 13−22.
(7) Fernandez-Lorente, G.; Godoy, C. A.; Mendes, A. A.; Lopez-Gallego, F.; Grazu, V.; de las Rivas, B.; Palomo, J. M.; Hermoso, J.; Fernandez-Lafuente, R.; Guisan, J. M. *Biomacromolecules* **2008**, *9* (9), 2553−2561.
(8) Palomo, J. M.; Fernandez-Lorente, G.; Mateo, C.; Ortiz, C.; Fernandez-Lafuente, R.; Guisan, J. M. *Enzyme Microb. Technol.* **2002**, *31* (6), 775−783.
(9) López-Gallego, F.; Abian, O.; Guisán, J. M. *Biochemistry* **2012**, *51* (35), 7028−7036.
(10) Kocer, A.; Walko, M.; Meijberg, W.; Feringa, B. L. *Science* **2005**, *309* (5735), 755−758.
(11) Pozhidaeva, N.; Cormier, M.-E.; Chaudhari, A.; Woolley, G. A. *Bioconjugate Chem.* **2004**, *15* (6), 1297−1303.
(12) Rau, H. Photoisomerization of Azobenzenes. In *Photochemistry and Photophysics*; CRC Press Inc.: Boca Raton, FL, 1990; Vol. *2*, p 21.
(13) Aizawa, M.; Namba, K.; Suzuki, S. *Arch. Biochem. Biophys.* **1977**, *182* (1), 305−10.
(14) Harvey, J. H.; Trauner, D. *ChemBioChem* **2008**, *9* (2), 191−193.
(15) Liu, D.; Karanicolas, J.; Yu, C.; Zhang, Z.; Woolley, G. A. *Bioorg. Med. Chem. Lett.* **1997**, *7* (20), 2677−2680.
(16) Schierling, B.; Noël, A. J.; Wende, W.; Hien le, T.; Volkov, E.; Kubareva, E.; Oretskaya, T.; Kokkinidis, M.; Rompp, A.; Spengler, B.; Pingoud, A. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107* (4), 1361−1366.
(17) Giger, L.; Caner, S.; Obexer, R.; Kast, P.; Baker, D.; Ban, N.; Hilvert, D. *Nat. Chem. Biol.* **2013**, *9* (8), 494−498.
(18) Coelho, P. S.; Brustad, E. M.; Kannan, A.; Arnold, F. H. *Science* **2013**, *339* (6117), 307−310.
(19) Kohler, V.; Mao, J.; Heinisch, T.; Pordea, A.; Sardo, A.; Wilson, Y. M.; Knorr, L.; Creus, M.; Prost, J. C.; Schirmer, T.; Ward, T. R. *Angew. Chem.* **2011**, *50* (46), 10863−10866.
(20) Hyster, T. K.; Knörr, L.; Ward, T. R.; Rovis, T. *Science* **2012**, *338* (6106), 500−503.
(21) Acosta, A.; Filice, M.; Fernandez-Lorente, G.; Palomo, J. M.; Guisan, J. M. *Bioresour. Technol.* **2011**, *102* (2), 507−512.
(22) Barbe, S.; Lafaquière, V.; Guieysse, D.; Monsan, P.; Remaud-Siméon, M.; André, I. *Proteins: Struct., Funct., Bioinf.* **2009**, *77* (3), 509−523.
(23) Lafaquière, V.; Barbe, S.; Puech-Guenot, S.; Guieysse, D.; Cortés, J.; Monsan, P.; Siméon, T.; André, I.; Remaud-Siméon, M. *ChemBioChem* **2009**, *10* (17), 2760−2771.
(24) Yamada, M. D.; Nakajima, Y.; Maeda, H.; Maruta, S. *J. Biochem.* **2007**, *142* (6), 691−698.
(25) Erbas-Cakmak, S.; Bozdemir, O. A.; Cakmak, Y.; Akkaya, E. U. *Chem. Sci.* **2013**, *4* (2), 858−862.
(26) Balzani, V.; Credi, A.; Venturi, M. *Molecular Devices and Machines: Concepts and Perspectives for the Nanoworld*, 2nd ed.; Wiley-VCH Verlag: Weinheim, 2008.