Supporting Information

Photo-controlled deactivation of immobilized Lipase

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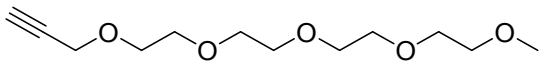
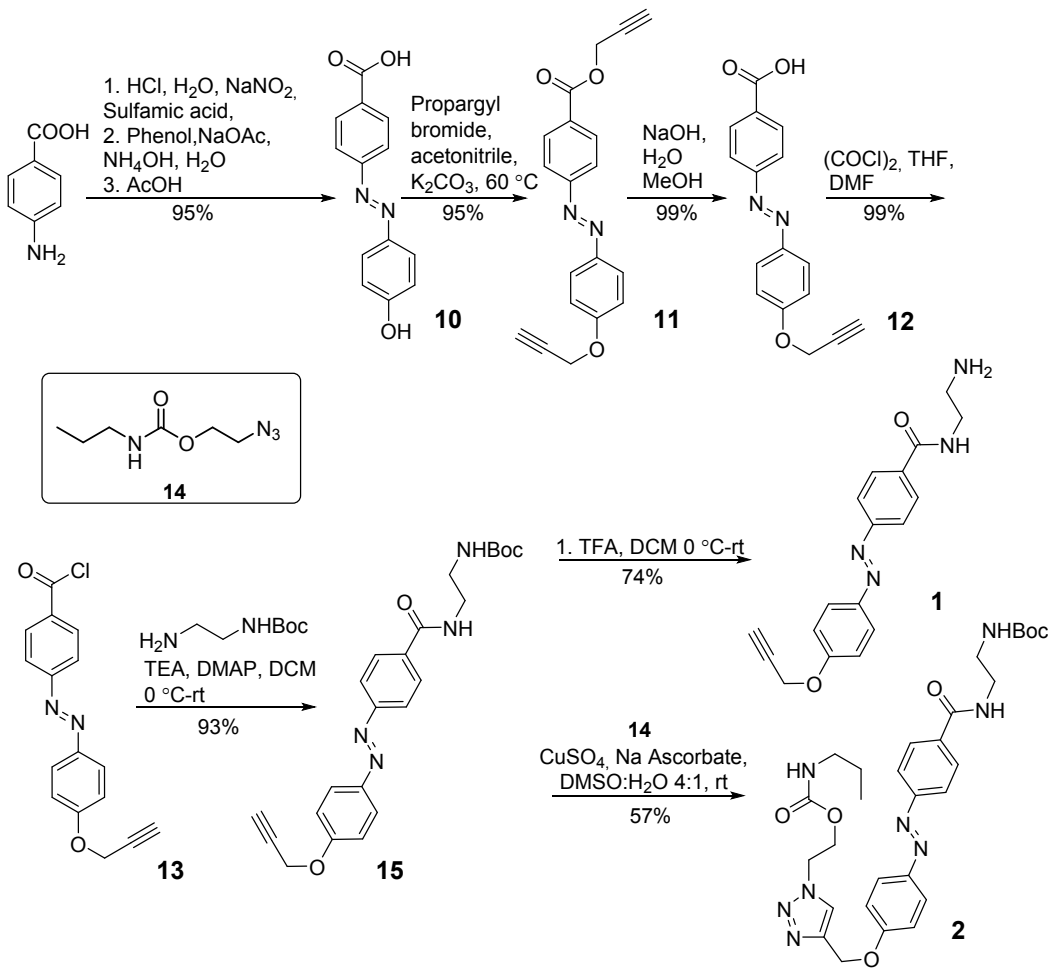
1. Synthesis of azobenzenes 1 and 2

All chemicals were obtained from commercial sources and used as received unless stated otherwise. Lipase from *Candida Rugosa* (lyophilized, fine powder, 15-25 U/mg) was obtained from Sigma Aldrich. Solvents were reagent grade. Thin-layer chromatography (TLC) was performed using commercial Kieselgel 60, F254 silica gel plates, and components were visualized with $KMnO_4$ or phosphomolybdic acid reagent. Flash chromatography was performed on silica gel (Silicycle Siliaflash P60, 40-63 m, 230-400 mesh). Drying of solutions was performed with $MgSO_4$ or Na_2SO_4 and solvents were removed with a rotary evaporator. Chemical shifts for NMR measurements were determined relative to the residual solvent peaks ($CHCl_3$, δ = 7.26 ppm for hydrogen atoms, δ = 77.0 for carbon atoms). The following abbreviations are used to indicate signal multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad signal. HRMS (ESI) spectra were obtained on a Thermo scientific LTQ Orbitrap XL. Melting points were recorded using a Buchi melting point B-545 apparatus. UV/Vis absorption spectra were recorded on an Agilent 8453 UV-Visible Spectrophotometer using Uvasol grade solvents. Irradiation experiments were performed with a spectroline ENB-280C/FE UV lamp (365 nm) and Thor Labs OSL1-EC Fiber Illuminator (white light). Irradiation at λ=400 nm was performed with a LED system (3 x 330 mW, λmax=401 nm, FWHM 13.5 nm, Sahlmann Photochemical Solutions).



6: 2,5,8,11,14-pentaoxaheptadec-16-yne was prepared according to a literature procedure.[1]

[1] B. A. Scates, B. L. Lashbrook, B. C. Chastain, K. Tominaga, B. T. Elliott, N. J. Theising, T. A. Baker, R. W. Fitch, *Bioorg. Med. Chem.*, 2008, **16**, 10295.



10: 4-(4-Hydroxyphenylazo)benzoic acid (modification of a literature procedure).[2] To an ice-cold solution of 4-aminobenzoic acid (17.5 mmol, 2.40 g) in 1.8N aqueous HCl (35 mL) was added dropwise a cold solution of $NaNO_2$ (17.5 mmol, 1.29 g) in water (2.5 mL). The yellow mixture was stirred at 0-5 °C for 1 h under a N_2 atmosphere. Sulfamic acid (160 mg) was added and the mixture was added dropwise to a cold solution of phenol (17.5 mmol, 1.66 g) and sodium acetate (2.41 g) in 1.0 M aq. NH_4OH (33 mL). The cooling bath was removed and the reaction mixture was stirred at rt for 1 h. Acetic acid (3.0 mL) was added and the precipitate was filtered off and washed with cold acetone, yielding **10** (4.02 g, 16.60 mmol, 95%). Red solid. ^{1}H NMR (400 MHz, CD_3OD): δ 6.93 (d, ^3J = 8.8 Hz, 2H, Ar**H**), 7.86 (d, ^3J = 8.8 Hz, 2H, Ar**H**), 7.89 (d, ^3J = 8.4 Hz, 2H, Ar**H**), 8.15 (d, ^3J = 8.4 Hz, 2H, Ar**H**); ^{1}H NMR spectrum in agreement with published data.[2]

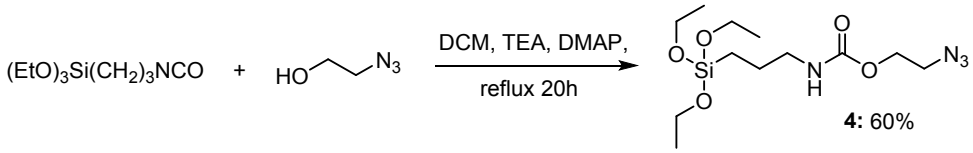
[2] S. Leclair, L. Mathew, M. Giguère, S. Motallebi, Y. Zhao, *Macromolecules*, 2003, **36**, 9024.

12: 4-(4-Propargyloxyphenylazo)benzoic acid. 4-(4-Hydroxyphenyl-azo)benzoic acid (**10**) (2.07 mmol, 500 mg) was dissolved in acetonitrile (20 mL). Propargyl bromide (8.00 mmol, 860 µL, as a 80% solution in toluene) was added, followed by potassium carbonate (6.00 mmol, 828 mg). The mixture was heated at reflux overnight. The volatiles were evaporated and the residue was dissolved in 1N aq. HCl (40 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic phases were dried ($MgSO_4$) and filtered through a plug of silicagel to afford, upon evaporation of the solvent, product **11** (623 mg, 95%); orange powder. R_f = 0.68 (pentane/AcOEt, 7:3, v/v); Mp. 127-128 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.54 (t, ^4J = 2.0 Hz, 1H, $CH_2CC\mathbf{H}$), 2.57 (t, ^4J = 2.4 Hz, 1H, $CH_2CC\mathbf{H}$), 4.79 (d, ^4J = 2.0 Hz, 2H, $C\mathbf{H_2}CCH$), 4.96 (d, ^4J = 2.4 Hz, 2H, $C\mathbf{H_2}CCH$), 7.11 (d, ^3J = 8.8 Hz, 2H, Ar**H**), 7.92 (d, ^3J = 8.8 Hz, 2H, Ar**H**), 7.96 (d, ^3J = 8.8 Hz, 2H, Ar**H**), 8.21 (d, ^3J = 8.8 Hz, 2H, Ar**H**); Product **11** was used immediately in the next step. Compound **11** (63 µmol, 20 mg) was dissolved in a mixture of methanol (1.8 mL) and tetrahyrofuran (1.8 mL). Aqueous sodium hydroxide (1N, 1.5 mL) was added and the mixture was stirred at rt for 2 h. The volatiles were evaporated, the residue was dissolved in aqueous HCl (1N, 20 mL) and extracted with ethyl acetate (2 x 20 mL). The organic phases were collected, dried and the solvent was evaporated to yield product **12** (17.5 mg, 99%); orange powder. R_f = 0.10 (pentane/Et_2O, 1:1, v/v); Mp. 230-231 °C; ^{1}H NMR (400 MHz, acetone-d_6): δ 3.17 (t, ^4J = 2.4 Hz, 1H, $CH_2CC\mathbf{H}$), 4.95 (d, ^4J = 2.4 Hz, 2H, $C\mathbf{H_2}CCH$), 7.23 (d, ^3J = 9.2 Hz, 2H, Ar**H**), 7.98 (d, ^3J = 8.8 Hz, 2H, Ar**H**), 8.01 (d, ^3J = 9.2 Hz, 2H, Ar**H**), 8.22 (d, ^3J = 8.8 Hz, 2H, Ar**H**); ^{13}C NMR (50 MHz, acetone-d_6): δ 55.8, 76.9, 114.7, 115.4, 122.3, 124.9, 130.7, 131.9, 147.3, 155.2, 160.9, 166.1; HRMS (ESI-) calc. for $C_{16}H_{11}N_2O_3$: 279.0768, found: 279.0764.

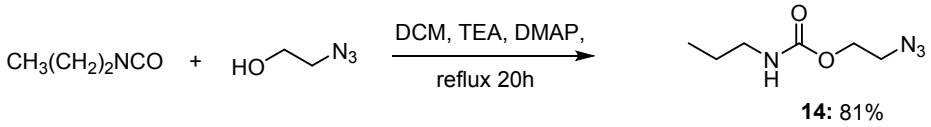
15: *Tert*-butyl (2-(4-((4-(propargyloxy)phenylazo)benzamido)ethyl)carbamate. A solution of 4-(4-propargyloxyphenylazo)benzoic acid (**12**) (0.50 mmol, 140 mg) and DMF (40 µL) in THF (3.0 mL) was stirred under a N_2 atmosphere at 0 °C. Oxalyl chloride (4.00 mmol, 340 µL) was added dropwise. When the evolution of gas ceased, the cooling bath was removed and the reaction mixture was stirred at rt for an additional 1 h. Evaporation of volatiles followed by drying *in vacuo* yielded compound **13** (148 mg, 99%) as a red solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.64 (t, ^4J = 2.4 Hz, 1H, $CH_2CC\mathbf{H}$), 4.93 (d, ^4J = 2.4 Hz, 2H, $C\mathbf{H_2}CCH$), 7.19 (d, ^3J = 9.2 Hz, 2H, Ar**H**), 7.90 (d, ^3J = 8.4 Hz, 2H, Ar**H**), 7.93 (d, ^3J = 9.2 Hz, 2H, Ar**H**), 8.10 (d, ^3J

= 8.4 Hz, 2H, Ar**H**). Compound **13** was used in the next step without further purification. To an ice-cooled solution of 4-(4-propargyloxyphenylazo)benzoyl chloride (**13**) (0.50 mmol, 149 mg) in DCM (10 mL) was added *N*-Boc-diaminoethane (1.00 mmol, 160 mg) followed by triethylamine (1.0 mmol, 140 μL) and DMAP (5 mg). The cooling bath was removed and the reaction mixture was stirred at rt for 1 h. The solvent was evaporated and the product **15** was purified by flash chromatography (Silicagel, 40-63 μm, pentane/AcOEt, 9:1 to 4:6, v/v) to yield 0.46 mmol (93%) as a red powder. Mp. 163-164 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 9H, (C**H$_3$**)$_3$), 2.57 (t, ^4J = 2.4 Hz, 1H, CH_2CC**H**), 3.36-3.48 (m, 2H, C**H$_2$**NH), 3.52-3.62 (m, 2H, C**H$_2$**NH), 4.77 (d, ^4J = 2.4 Hz, 2H, C**H$_2$**CCH), 5.14 (br s, 1H, N**H**Boc), 7.08 (d, ^3J = 8.8 Hz, 2H, Ar**H**), 7.44 (br s, 1H, N**H**), 7.88 (d, ^3J = 8.8 Hz, 2H, Ar**H**), 7.93 (d, ^3J = 9.2 Hz, 2H, Ar**H**), 7.95 (d, ^3J = 8.4 Hz, 2H, Ar**H**); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.6, 40.1, 42.6, 56.3, 76.3, 78.2, 80.3, 115.4, 122.8,125.2, 128.2, 135.7, 147.7, 154.6, 157.9, 160.5, 167.4, 177.8; HRMS (ESI+) calc. for $C_{23}H_{26}N_4O_4$: 445.1846, found: 445.1851.

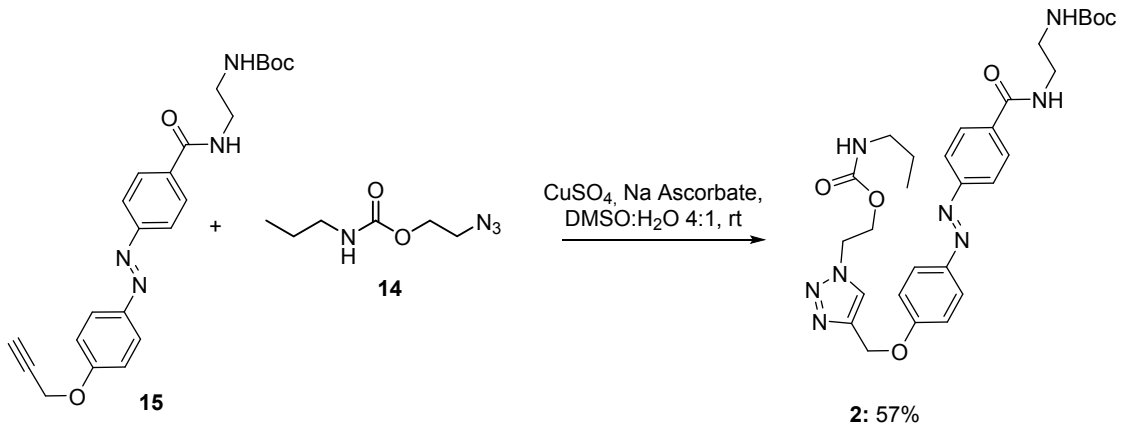
1: *N*-(2-aminoethyl)-4-((4-(prop-2-yn-1-yloxy)phenyl)diazenyl)benzamide. To an ice-cooled solution of tert-butyl (2-(4-((4-(propargyloxy)phenylazo)benzamido)-ethyl)carbamate (**15**) (0.46 mmol, 196 mg) in DCM (7.0 mL) was added TFA (1.0 mL). The cooling bath was removed and the reaction mixture was stirred for 90 min at rt. The solvent was evaporated and the product was purified by flash chromatography (Silicagel, 40-63 μm, AcOEt to AcOEt/MeOH 9:1, v/v) to yield 110 mg (0.34 mmol,74%) of a red oil. ^{1}H NMR (400 MHz, acetone-d_6): δ 1.19 (t, ^3J = 7.2 Hz, 2H, N**H$_2$**), 3.18 (t, ^3J = 2.4 Hz, 2H, CC**H**), 3.82-3.94 (m, 2H, C**H$_2$**NH), 4.02-4.07 (m, 2H, C**H$_2$**NH$_2$), 4.94 (d, ^4J = 2.4 Hz, 2H, C**H$_2$**CCH), 7.20 (d, ^3J = 8.8 Hz, 2H, Ar**H**), 7.91 (d, ^3J = 7.2 Hz, 2H, Ar**H**), 7.98 (d, ^3J = 8.8 Hz, 2H, Ar**H**), 8.17 (d, ^3J = 7.2 Hz, 2H, Ar**H**); 9.41 (t, ^3J = 4.8 Hz, 1H, CH_2N**H**); ^{13}C NMR (75 MHz, acetone-d_6): δ 37.3, 47.2, 55.8, 76.7, 78.3, 113.7, 114.7, 115.4, 122.2, 124.8, 128.6, 147.2, 154.2, 160.7; HRMS (ESI+) calc. for $C_{18}H_{19}N_4O_2$: 323.1502, found: 323.1497.



4: 2-Azidoethyl (3-(triethoxysilyl)propyl)carbamate. A solution of 3-(triethoxysilyl)propyl isocyanate (5.00 mmol, 1.24 mL), 2-azidoethanol (5.20 mmol, 452 mg), triethylamine (12.5 mmol, 1.74 mL) and DMAP (10 mg) in DCM (7.0 mL) was heated at reflux for 20 h. The volatiles were evaporated, the residue was taken up in AcOEt (40 mL), washed rapidly with 1N aq. HCl (30 mL), saturated aq. $NaHCO_3$ (30 mL) and brine (30 mL). The organic phase was dried ($MgSO_4$) and the volatiles were evaporated to yield pure **4** as a slightly yellow oil (1.01 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61-0.66 (m, 2H, C**H**$_2$Si), 1.23 (t, 9H, ^3J = 6.8 Hz, C**H**$_3$CH$_2$), 1.60-1.68 (m, 2H, C**H**$_2$CH$_2$Si), 3.15-3.23 (m, 2H, C**H**$_2$NH), 3.45 (t, 2H, ^3J = 4.8 Hz, C**H**$_2$N$_3$), 3.82 (q, 6H, ^3J = 6.8 Hz, C**H**$_3$CH$_2$), 4.23 (t, 2H, ^3J = 4.8 Hz, C**H**$_2$O), 5.03 (br s, 1H, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$): 7.5, 18.2, 23.2, 43.4, 50.3, 58.4, 63.2, 155.8; HRMS (ESI+) calc. for $C_{12}H_{27}N_4O_5SiNa$: 357.1570, found: 357.1560.



14: 2-azidoethyl propylcarbamate. A solution of propyl isocyanate (5.00 mmol, 469 µL), 2-azidoethanol (5.20 mmol, 452 mg), triethylamine (12.5 mmol, 1.74 mL) and DMAP (10 mg) in DCM (7.0 mL) was heated at reflux for 20 h. The volatiles were evaporated, the residue was taken up in Et_2O (30 mL), washed rapidly with 1N aq. HCl (3 x 25 mL), aq. saturated $NaHCO_3$ (2 x 25 mL) and brine (25 mL). The organic phase was dried ($MgSO_4$) and the volatiles were evaporated to yield pure compound **14** as a slightly yellow oil (700 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (t, 3H, ^3J = 7.6 Hz, C**H**$_3$CH$_2$), 1.49-1.58 (m, 2H, C**H**$_2$CH$_3$), 3.13-3.20 (m, 2H, C**H**$_2$NH), 3.44-3.48 (m, 2H, C**H**$_2$N$_3$), 4.22-4.28 (m, 2H, C**H**$_2$O), 4.81 (br s, 1H, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$): 11.1, 23.1, 42.8, 50.3, 63.3; HRMS (ESI+) calc. for $C_6H_{13}N_4O_2$: 173.1033, found: 173.1033.



2: (E)-tert-butyl (2-(4-((4-((1-(2-((propylcarbamoyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)diazenyl)benzamido)ethyl)carbamate. A pre-incubated (10 min) mixture of $CuSO_4$ (0.050 mmol, 12.5 mg) and sodium ascorbate (0.10 mmol, 20 mg) in water (1.0 mL) was added to a stirred solution of compound **15** (0.26 mmol, 110 mg) and compound **14** (0.50 mmol, 86 mg) in DMSO (3.0 mL). After stirring overnight at rt, the reaction mixture was diluted with AcOEt (100 mL) and washed with water (50 mL) and brine (3 x 50 mL). The organic phase was dried ($MgSO_4$) and the volume of the sample was reduced to ~20 mL. Addition of pentane (40 mL) resulted in a formation of a precipitate which was filtered off, washed with pentane and Et_2O and dried to yield product **2** (44 mg, 57%) as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.80 (t, 3H, ^3J = 7.2 Hz, C**H$_3$**CH$_2$), 1.32-1.38 (m, 2H, C**H$_2$**CH$_3$), 1.36 (s, 9H, (C**H$_3$**)$_3$), 2.85-2.88 (m, 2H, CH$_3$CH$_2$C**H$_2$**), 3.08-3.12 (m, 2H, C**H$_2$**NH), 3.27-3.31 (m, 2H, C**H$_2$**NH), 4.34 (t, 2H, ^3J = 5.2 Hz, CH$_2$C**H$_2$**triazole), 4.61 (t, ^3J = 5.2 Hz, 2H, C**H$_2$**CH$_2$triazole), 5.27 (s, 2H, OC**H$_2$**triazole), 6.88-6.93 (m, 1H, N**H**Boc), 7.20 (t, ^3J = 5.2 Hz, 1H, CH$_3$CH$_2$CH$_2$N**H**), 7.24 (d, ^3J = 9.2 Hz, 2H, Ar**H**), 7.90 (d, ^3J = 8.4 Hz, 2H, Ar**H**), 7.93 (d, ^3J = 9.2 Hz, 2H, Ar**H**), 8.00 (d, ^3J = 8.4 Hz, 2H, Ar**H**), 8.28 (s, 1H, traizole**H**), 8.59 (t, ^3J = 5.2 Hz, 1H, N**H**Boc); ^{13}C NMR (100 MHz, DMSO-d_6):δ 11.6, 23.0, 28.7, 40.2, 40.4, 42.1, 49.7, 62.0, 62.5, 78.1, 115.9, 122.7, 125.3, 128.9, 131.0, 136.5, 142.7, 146.8, 153.8, 156.0, 156.2,161.6, 166.1; HRMS (ESI+) calc. for $C_{29}H_{39}N_8O_6$: 595.2992, found: 595.2984. UV-Vis (20 x 10^{-6} M in MeOH): λ_{max} = 352 nm, ε_{352} = 19.7 x 10^3 M^{-1} cm^{-1}.

2. PSS determination of 2 by ^{1}H-NMR

A saturated solution of compound **2** in CD_3OD was prepared and the ^{1}H-NMR spectrum shows >97% *trans*2 (Fig. 1a). The solution in the NMR tube was irradiated for 30 min with λ = 365 nm light and the ^{1}H-NMR was spectrum shows >95% *cis*–**2** (Fig. 1b).

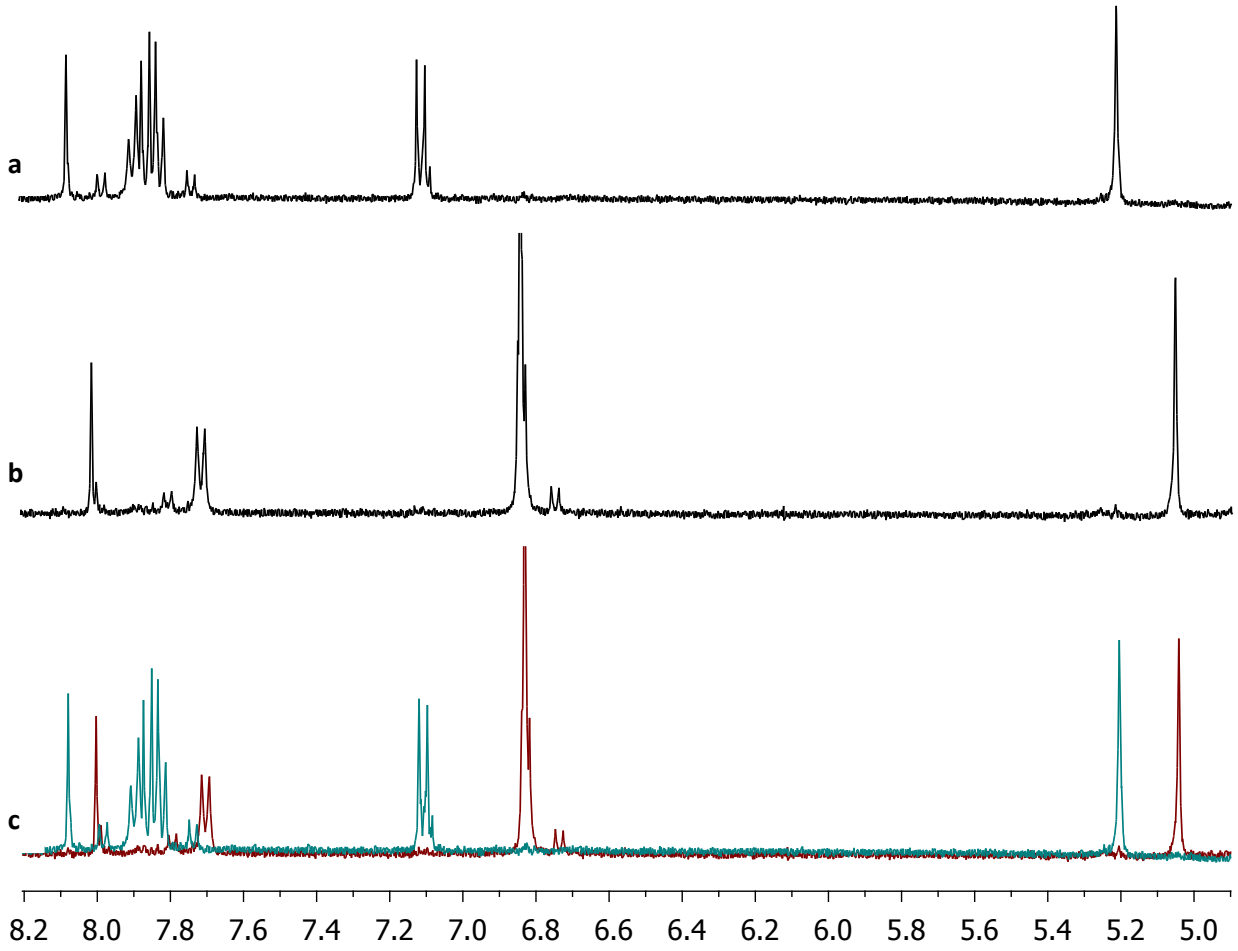


Fig. 1: a) Aromatic region of ^{1}H-NMR spectrum for thermally adapted >97% *trans*–**2.** b) Aromatic region of ^{1}H-NMR spectrum for PSS-mixture with >95% *cis* – **2.** c) Superimposed ^{1}H-NMR spectra *trans*–**2** and *cis*–**2.** Solvent: CD_3OD.

3. UV-Vis studies on compound 2

A solution of compound **2** (20 x 10^{-6} M solution in MeOH) was irradiated with white light for 1 min and the UV-vis absorption spectrum was recorded (Fig. 2a). Then the same solution was irradiated with λ = 365 nm light for 1 min (Fig. 2), while the absorbance spectrum was recorded every 10 sec.

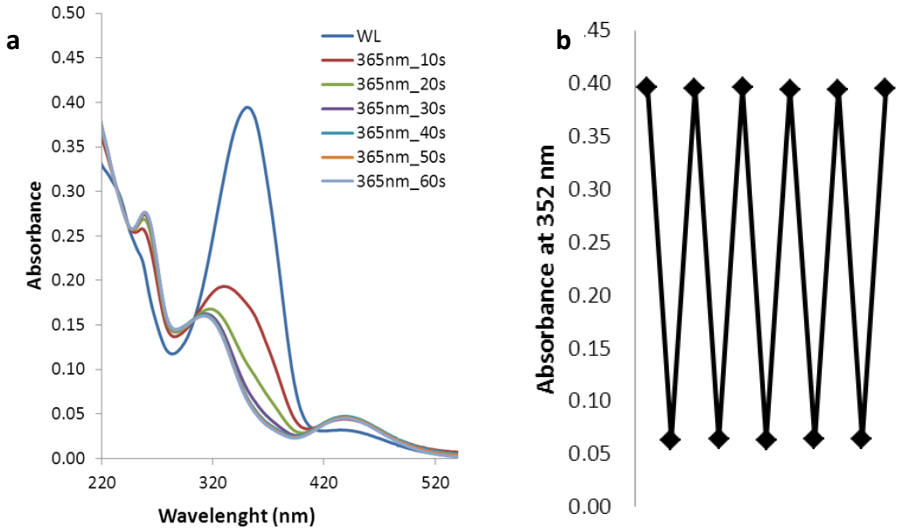


Fig. 2: a) UV-vis spectra of compound **2** (20 x 10^{-6} M solution in MeOH) after 1 min white light irradiation, and after every 10 sec of λ = 365 nm irradiation. b) Switching cycles of compound **2.**

A solution of compound **2** (20 x 10^{-6} M M solution in MeOH) was irradiated at 365 nm for 1 min. The change in absorbance at 352 nm was subsequently measured. The increase of absorbance at 352 nm was fitted with a single exponential curve.

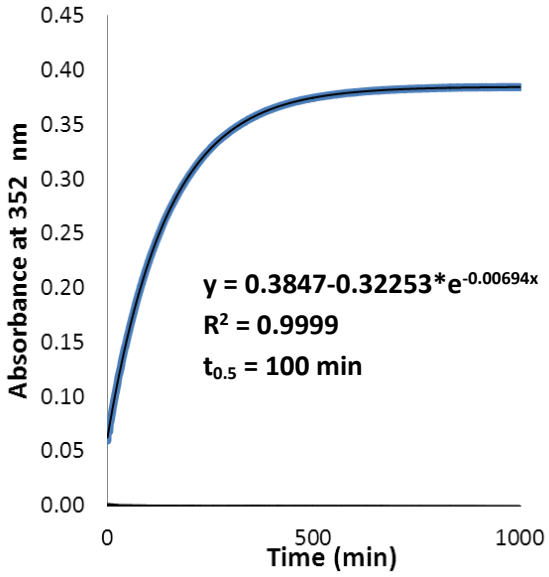
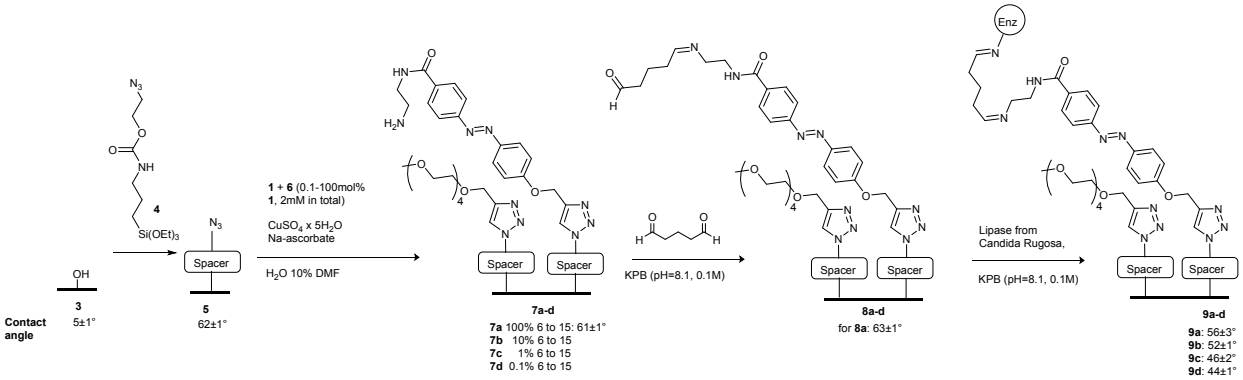


Fig. 3: Kinetic data for the *cis* - *trans* isomerization. Blue line represents the measured changes in absorbance, black line represents the fitting with simple exponential decay.

4. Surface modification



CAUTION: Piranha solution, used in this procedure, is potentially explosive. It must be handled with extreme caution. Disposal: Piranha solution should not be stored in a closed container. Piranha solution must not be disposed with organic solvents. Solution must be cooled down to room temperature prior to waste storage. It has to be diluted by pouring into excess water before disposal.

Quartz microscopy coverslips were cleaned using piranha solution (conc. H_2SO_4 : 30% H_2O_2, 3:1, v/v), rinsed with water (2x) and MeOH (2x), dried under N_2 stream and cleaned by a plasma cleaner (1 min, 100% O_2, 1.5 mbar).

Contact angle measurements were recorded on a Data Physics contact angle goniometer and the contact angle was calculated using the software provided by the company. The reported data are the average of the contact angles measured for three drops for every quartz coverslip.

5: Azide-functionalized quartz coverslips was prepared according to a previously published procedure.[3]

7: Azide-functionalized quartz coverslips were immersed in a 2 mM solution comprising a mixture of compounds **1** and **6** (from 0.1 mol% to 100 mol% **1**) and 1 mol% $CuSO_4 \cdot 5H_2O$ and 5 mol% sodium ascorbate relative to alkyne moieties, in DMF/H_2O (9:1). After 1 d the coverslips were sonicated in DMF and MeOH (1 min each), and then dried under a N_2 stream.

8: Azobenzene-functionalized quartz coverslips were immersed in 10% v/v solution of glutaraldehyde in phosphate buffer (0.1M, pH 7.9-8.1) at rt. After 1 d the coverslips were sonicated in water (2x) and then dried under a N_2 stream.

9: Glutaraldehyde-functionalized quartz coverslips were immersed in a solution of Lipase from *Candida rugosa* (13 mg in 10 mL phosphate buffer 0.1M, pH 7.9-8.1) at rt. After 1d the coverslips were sonicated in water (5x) and then dried under N_2 stream.

5. UV-vis studies of the surface assembly

[3] Szymanski W., Wu B., Poloni C., Janssen D. B., Feringa B. L., Angew. Chem. Int. Ed. **2013**, *52*, 2068 –2072

UV-vis spectroscopy measurements were carried out on dried quartz coverslips using a slip holder in an Agilent 8453 UV-Visible spectrophotometer. Irradiation with λ = 365 nm and white light was carried out without removing the slips from the holder.

The coverslips were irradiated in cycles at λ = 365 nm for 5 min and white light for 2 min.

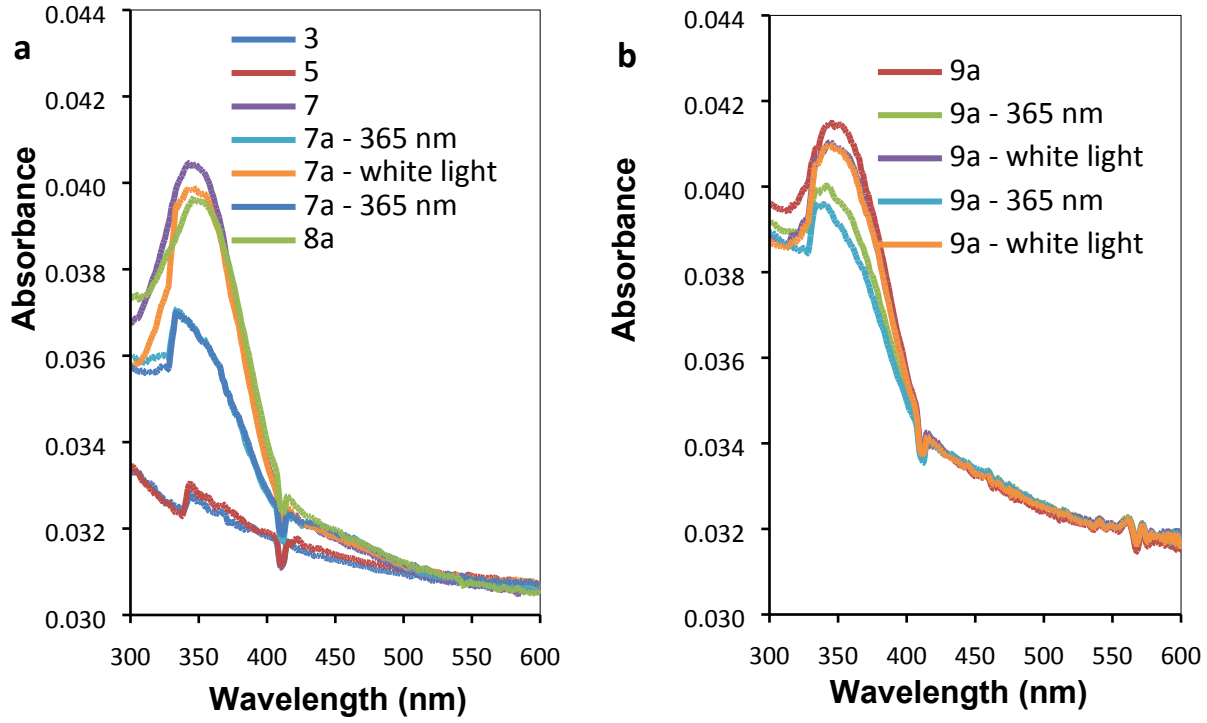


Fig. 4: a) UV-vis spectra of cleaned coverslip **3**, azide-functionalized coverslips **5**, azobenzene-functionalized coverslip **7a** and its changes of UV-vis absorption spectrum during the subsequent λ = 365 nm and white light irradiation, and glutaraldehyde-functionalized coverslip **8**. b) UV-vis absorption spectra of lipase-functionalized coverslip **9a** and its changes of absorption after the subsequent λ = 365 nm and white light irradiation.

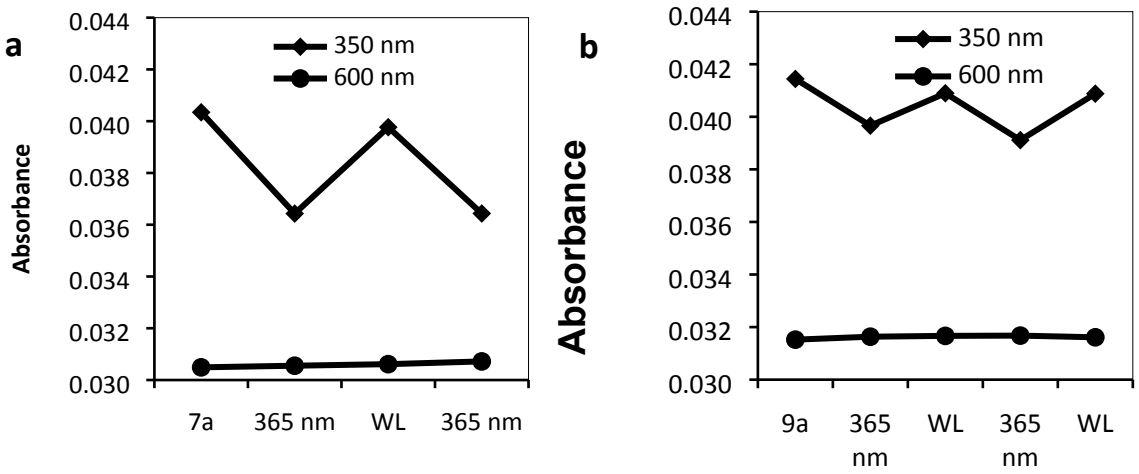


Fig. 5: a) Switching cycles of **7a**, and b) lipase-functionalized coverslip **9a**. Changes in absorbance were measured at 350 nm and at 600 nm (background).

6. Activity assay for lipase-functionalized coverslips

0.6 mL of 10 mM solution of *p*-nitrophenyl butyrate in *iso*-propanol was mixed with 5.4 mL TRIS HCl buffer (50 mM, pH 7.5). Subsequently, the lipase-functionalized coverslip was immersed in the solution. Every minute the solution was removed and its absorbance at λ=442 nm was recorded (ε = 3.07 mM^{-1} cm^{-1}). The hydrolytic activity of the lipase-functionalized coverslip was corrected for the non-catalyzed hydrolysis of *p*-nitrophenyl butyrate in buffer. After the enzymatic assay, the cover slip was rinsed with milliQ water. Next, the coverslip was irradiated with λ = 365 nm or white light and the enzymatic assay was repeated. The irradiation at λ = 365 nm was performed with dry coverslips for 1 h and white light irradiation was performed with dry coverslips for 5 min. For **9c**, the assay was repeated for at least 3 functionalized quartz coverslips (Table 2).

Table 1: Enzymatic activity (coverslip area-normalized) for lipase-functionalized coverslips **9a** to **9d**.

u (mM cm^{-2} min^{-1})	**9a**	**9b**	**9c**	**9d**
λ = 365 nm	2.4E-04	2.3E-04	2.56E-04	1.58E-05
White light	2.11E-04	9.96E-05	4.23E-05	1.16E-05

Table 2. Enzymatic activity (mM cm^{-2} min^{-1}) for lipase-functionalized coverslips **9c**.

Wash	λ = 365 nm	White light	λ = 365 nm (after white light irradiation)
3.73E-04	1.93E-04	5.48E-05	1.7E-06
3.49E-04	3.19E-04	2.99E-05	3.82E-05
3.00E-04	1.7E-04	5.00 E-06	2.5E-05
4.4E-05			
3.57E-04			
Average	**Average**	**Average**	**Average**
(2.8±1.4)E-04	**(2.3±0.8)E-04**	**(0.4±0.1)E-04**	**(0.4±0.2)E-04**

7. Control experiments for enzymatic assay on lipase-functionalized coverslips

7.1. The first control experiment was aimed at establishing if irradiation with white light or light at λ = 365 nm influences the enzymatic activity in solution. For this purpose, the enzymatic activity of lipase in solution was measured, then λ = 365 nm or white light irradiation was applied and the activity was measured again. (Fig. 8)

Conditions: 0.3 mL of 10 mM solution of *p*-nitrophenyl butyrate in *iso*-propanol was mixed with 2.65 mL TRIS HCl buffer (50 mM, pH 7.5). 0.025 mL of enzyme solution (0.017 mg/mL) in milliQ water was added. Every 30 sec the absorbance at 442 nm was recorded (ε = 3.07 mM^{-1} cm^{-1}). This procedure was repeated after irradiation of the solution with λ = 365 nm for 5 min and then after white light irradiation for 5 min. The measured changes in absorbance at λ = 442 nm are presented in Fig. 8.

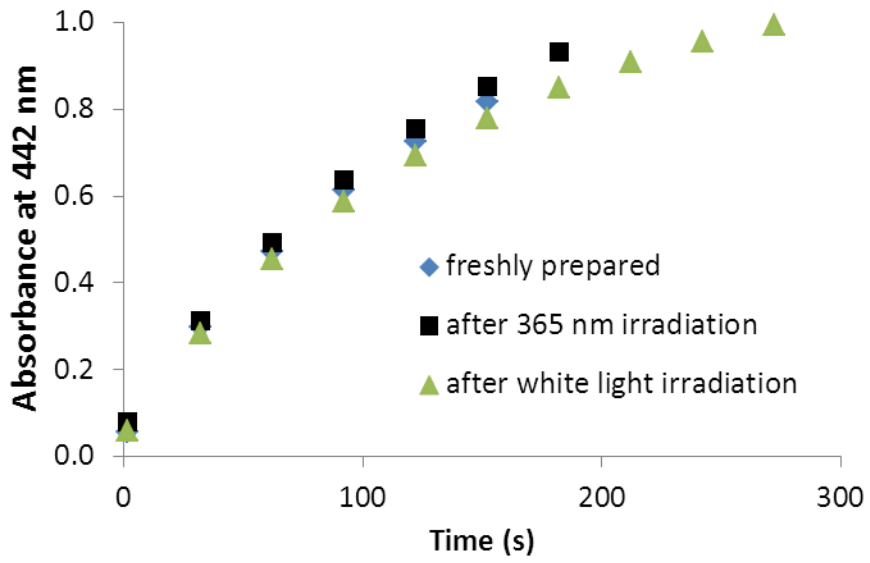


Figure 8: a) Enzymatic activity for lipase in solution (0.14 μg/mL, TRIS HCl buffer 50 mM, pH 7.5) before and after irradiation with white light and at λ = 365 nm light.

No significant change in activity was observed, which excludes the possible inactivation of the non-modified enzyme upon irradiation.

7.2 The second set of control experiments was aimed at checking if the irradiation results in the desorption of the enzyme from the immobilisate (Figure 9).

Conditions: Three samples were prepared, each containing 0.6 mL of 10 mM solution of *p*-nitrophenyl butyrate in *iso*-propanol and 5.4 mL TRIS HCl buffer (50 mM, pH 7.5). A functionalized coverslip was immersed in the enzymatic assay solution for 5 min, then removed and the absorbance of the solution at 442 nm was measured for 4 min. The same coverslip was irradiated at λ = 365 nm for 5 min, immersed for 5 min in a fresh enzymatic assay solution and then removed. The absorbance of the solution was measured again for 4 min. The same coverslip was irradiated with white light for 5 min, immersed for 5 min in a new enzymatic assay solution and then removed (Fig. 9a).

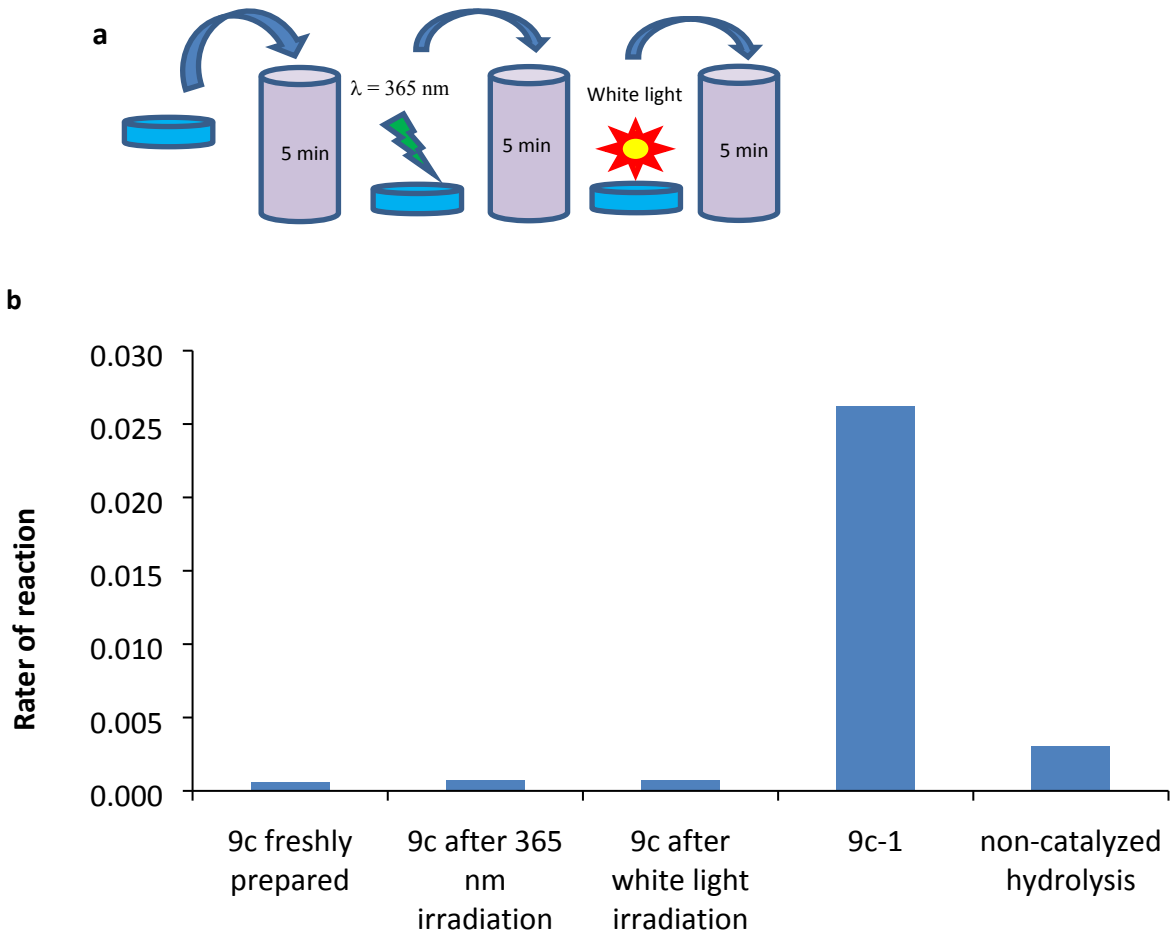


Figure 9: a) Schematic representation of the experimental setup. b) Rates of reaction for hydrolysis in the solutions from which the coverslips were removed for: freshly prepared **9c**, after λ = 365 nm irradiation, after white light irradiation, the rates of reaction for the catalyzed **9c** and non-catalyzed hydrolysis.

The observed residual activities in the solutions from which the non-irradiated and irradiated coverslips were removed are comparable to the rate of non-catalyzed hydrolysis (Fig. 9b). This observation indicates that the irradiation does not result in the desorption of the active biocatalyst from the coverslip.

8 Control experiment for enzymatic assay using a different irradiation source

A solution of compound **2** was prepared in methanol (c= 20 x 10^{-6} M). The sample was irradiated for 1 min at λ= 365 nm, then irradiated for 5 min at λ= 400 nm, then irradiated for 1 min with white light, again at λ= 365 nm and finally for 20 min at λ= 400 nm. The process was followed by UV-vis spectroscopy (Fig. 10).

The enzymatic assay, described previously, was repeated for coverslip **9c**. The coverslip was irradiated at λ= 365 nm for 1h and then at λ= 400 nm for 1 h. For the coveslip after ittadiation at λ= 365 nm, the enzymatic activity is $0{,}21 \cdot 10^{-3}$ mM cm^{-2} min^{-1} and for the coveslip after ittadiation at λ= 400 nm, the enzymatic activity is $0{,}15 \cdot 10^{-3}$ mM cm^{-2} min^{-1}(Fig. 11).

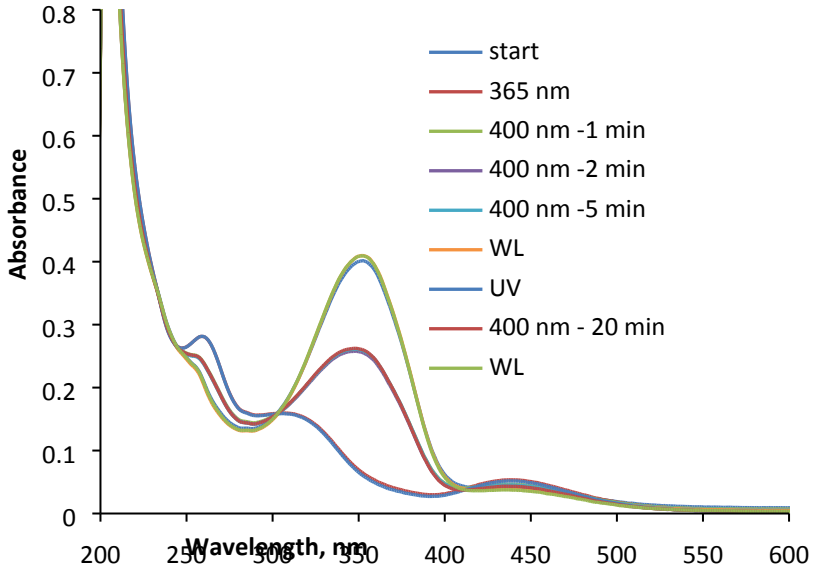


Figure 10: UV-vis absorption spectra for compound **2**, after irradiation at λ= 365 nm, after irradiation at λ= 400 nm for 1 min, 2 min, 5 min, 20 min and after irradiation with white light.

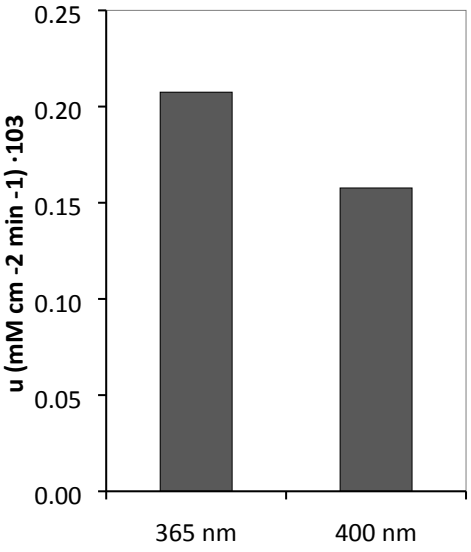


Figure 11: Enzyme activity for lipase immobilized coverslip after irradiation at λ= 365 nm and after irradiation at λ= 400 nm.