

**Photo-Controlling Enantioselectivity of
Phosphotriesterase via the Incorporation of a
Light-Responsive Non-Canonical Amino Acid**



Master`s Thesis

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submitted by

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1. Supplement

1.1 Time Course of Substrate Hydrolysis

1.1.1 Chemical Hydrolysis

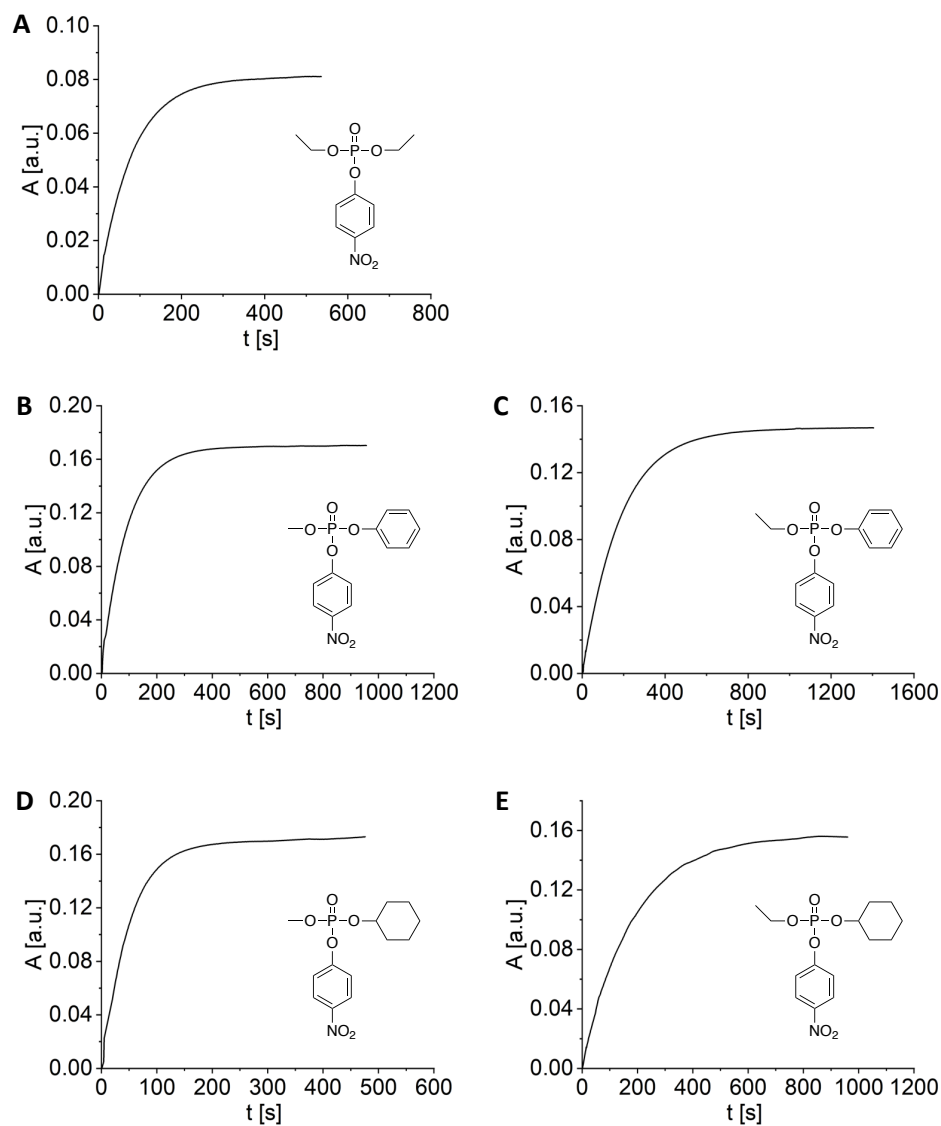


Figure S 1: Chemical hydrolysis of PTE substrates used in this work

Quantitative hydrolysis of substrates I-V by addition of KOH **A** Hydrolysis of 5 μM substrate I by addition of 1.5 M KOH. **B** Hydrolysis of 10 μM racemic substrate II by addition of 0.1 M KOH. **C** Hydrolysis of 10 μM racemic substrate III by addition of 0.1 M KOH. **D** Hydrolysis of 10 μM racemic substrate IV by addition of 3 M KOH. **E** Hydrolysis of 10 μM racemic substrate V by addition of 2 M KOH.

1.1.2 PTE_wt

Substrate I

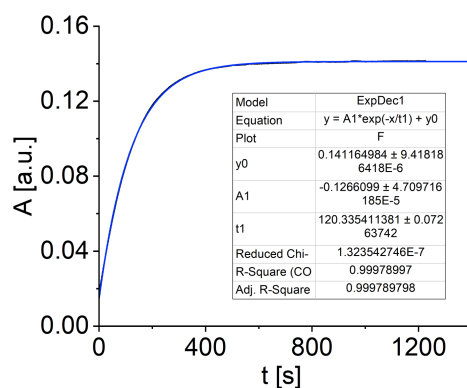


Figure S 2: Enzymatic hydrolysis of substrate I catalyzed by PTE_wt

Time course for the hydrolysis of 10 μM substrate I by addition of 0.1 nM PTE_wt (black). The data was fitted to a single exponential function (blue).

Substrate II

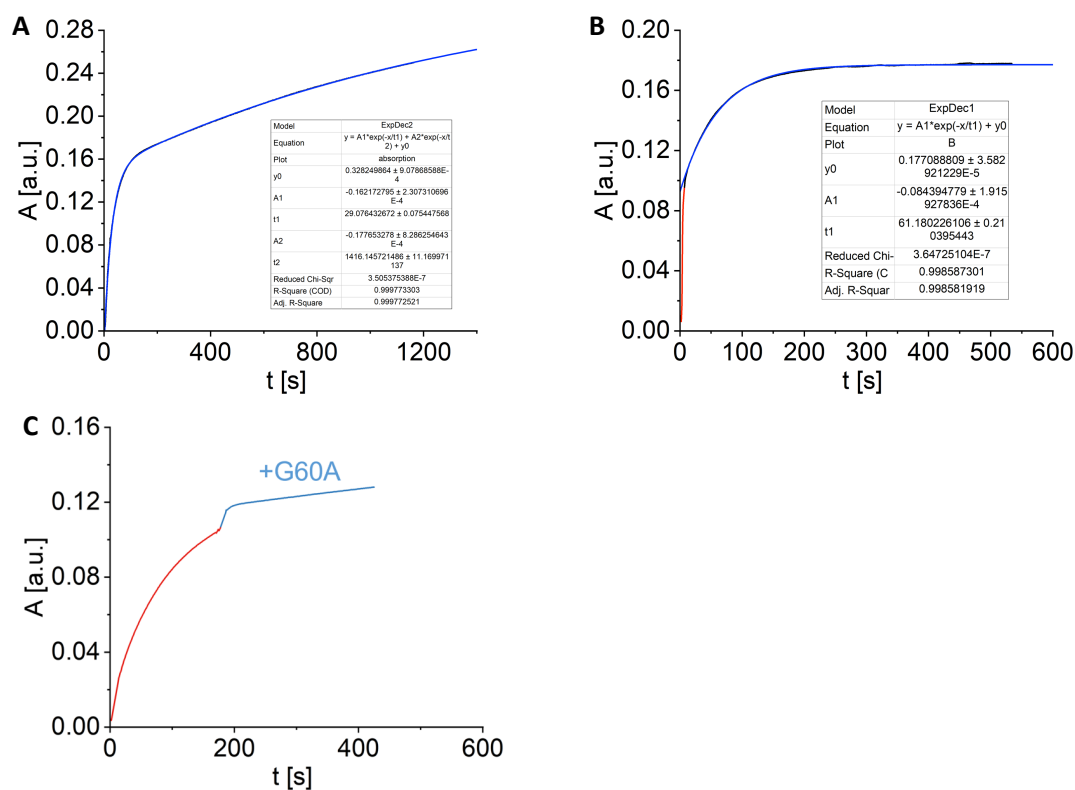


Figure S 3: Enzymatic hydrolysis of substrate II catalyzed by PTE_wt

A Time course for the hydrolysis of 20 μM II-S_p/R_p by 0.5 nM PTE_wt (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_wt for the hydrolysis of II-S_p. **B** Time course for the hydrolysis of 20 μM II-S_p/R_p by 5 nM PTE_wt (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_wt for the hydrolysis of II-R_p. Data points corresponding to the hydrolysis of II-S_p (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_wt. Enzymatic hydrolysis of 10 μM substrate II was initiated by addition of 0.5 nM PTE_wt and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that II-S_p is preferentially hydrolyzed.

Substrate III

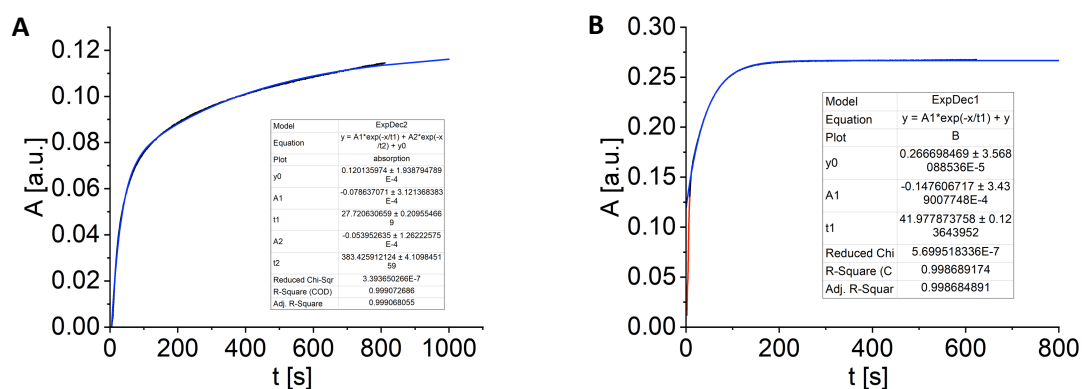


Figure S 4: Enzymatic hydrolysis of substrate III catalyzed by PTE_wt

A Time course for the hydrolysis of 20 μ M III-S_P/R_P by 0.5 nM PTE_wt (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_wt for the hydrolysis of III-S_P. **B** Time course for the hydrolysis of 20 μ M III-S_P/R_P by 5 nM PTE_wt (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_wt for the hydrolysis of III-R_P. Data points corresponding to the hydrolysis of II-S_P (red) were not considered in the fit.

Substrate IV

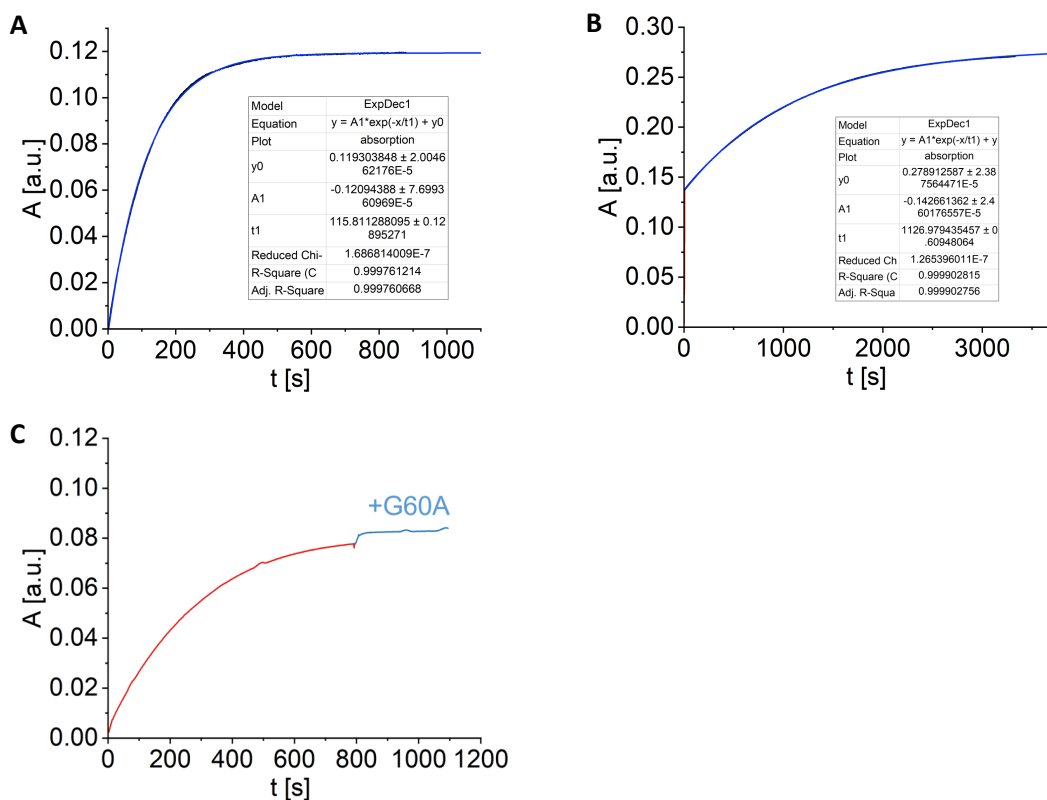


Figure S 5: Enzymatic hydrolysis of substrate IV catalyzed by PTE_wt

A Time course for the hydrolysis of 20 μM IV-S_p/R_p by 1 nM PTE_wt (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_wt for the hydrolysis of IV-S_p. **B** Time course for the hydrolysis of 20 μM IV-S_p/R_p by 2 μM PTE_wt (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_wt for the hydrolysis of IV-R_p. Data points corresponding to the hydrolysis of IV-S_p (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_wt. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 1 nM PTE_wt and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that IV-S_p is preferentially hydrolyzed.

Substrate V

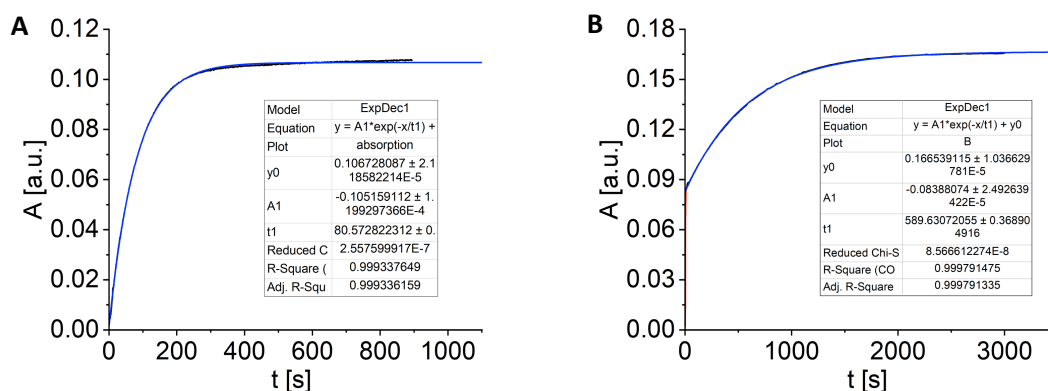


Figure S 6: Enzymatic hydrolysis of substrate V catalyzed by PTE_wt

A Time course for the hydrolysis of 20 μM V-S_P/R_P by 1 nM PTE_wt (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_wt for the hydrolysis of V-S_P. **B** Time course for the hydrolysis of 10 μM V-S_P/R_P by 4 μM PTE_wt (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_wt for the hydrolysis of V-R_P. Data points corresponding to the hydrolysis of V-S_P (red) were not considered in the fit.

1.1.3 PTE_G60A

Substrate I

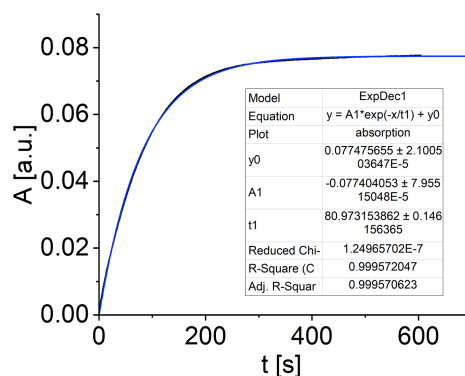


Figure S 7 Enzymatic hydrolysis of substrate I catalyzed by PTE_G60A

Time course for the hydrolysis of 5 μ M substrate I by addition of 2 nM PTE_G60A (black). The data was fitted to a single exponential function.

Substrate II

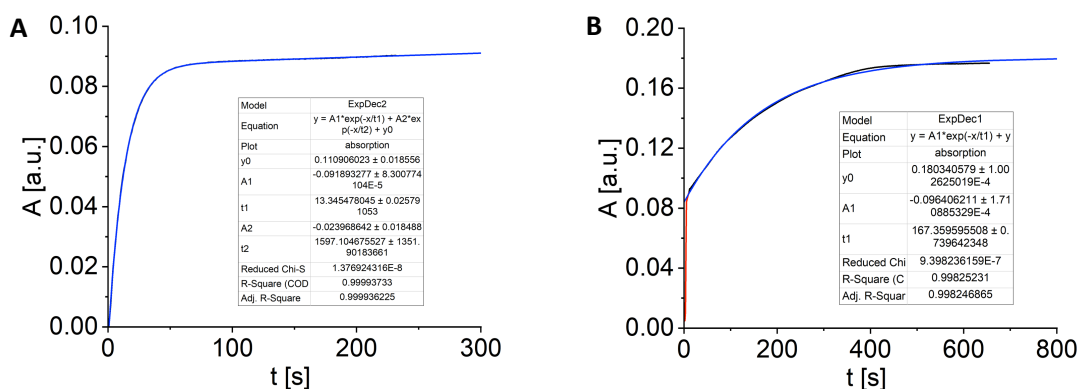


Figure S 8: Enzymatic hydrolysis of substrate II catalyzed by PTE_G60A

A Time course for the hydrolysis of 10 μ M II-S_p/R_p by 2 nM PTE_G60A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_G60A for the hydrolysis of II-S_p. **B** Time course for the hydrolysis of 10 μ M II-S_p/R_p by 1 μ M PTE_G60A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_G60A for the hydrolysis of II-R_p. Data points corresponding to the hydrolysis of II-S_p (red) were not considered in the fit.

Substrate III

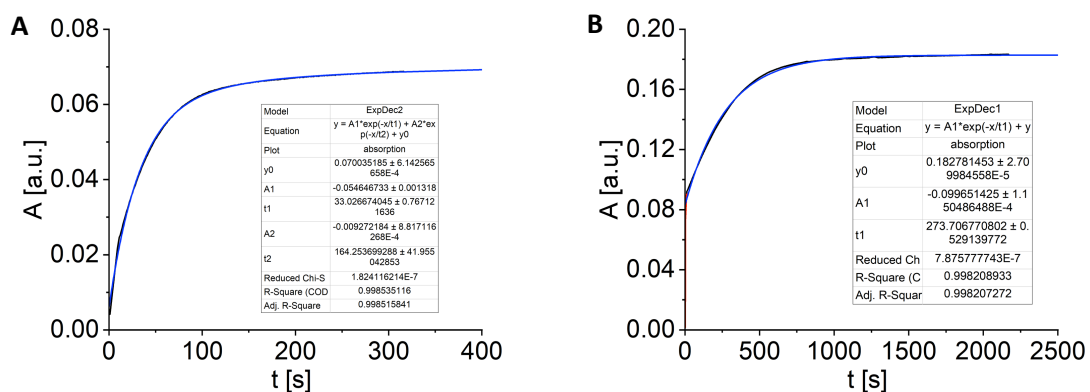


Figure S 9: Enzymatic hydrolysis of substrate III catalyzed by PTE_G60A

A Time course for the hydrolysis of 10 μ M III-S_p/R_p by 1 nM PTE_G60A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_G60A for the hydrolysis of III-S_p. **B** Time course for the hydrolysis of 10 μ M III-S_p/R_p by 1 μ M PTE_G60A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_G60A for the hydrolysis of III-R_p. Data points corresponding to the hydrolysis of II-S_p (red) were not considered in the fit.

Substrate IV

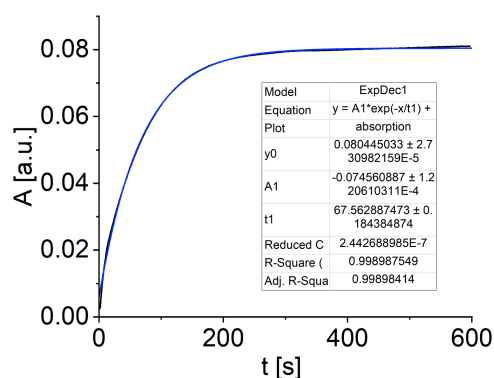


Figure S 10: Enzymatic hydrolysis of substrate IV catalyzed by PTE_G60A

A Time course for the hydrolysis of 10 μ M IV-S_p/R_p by 2 nM PTE_G60A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_G60A for the hydrolysis of IV-S_p.

Substrate V

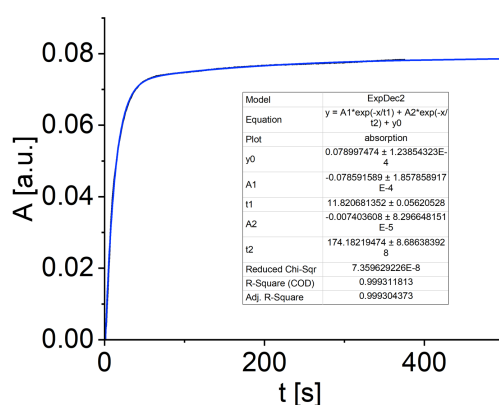


Figure S 11: Enzymatic hydrolysis of substrate V catalyzed by PTE_G60A

A Time course for the hydrolysis of 20 μM V-S_P/R_P by 5 nM PTE_G60A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_G60A for the hydrolysis of V-S_P.

1.1.4 PTE_H254ONBY

Substrate I

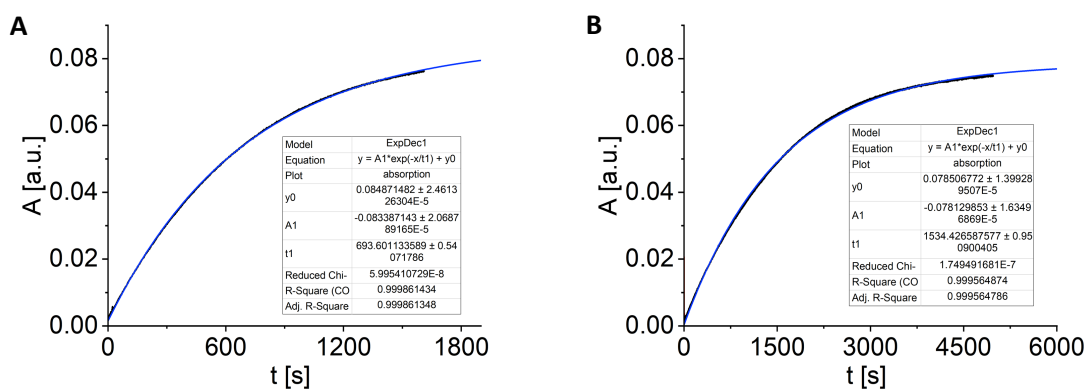


Figure S 12: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254ONBY before and after illumination

A Time course for the hydrolysis of 5 μM substrate I by 1 nM PTE_H254ONBY *as isolated* (black). The data was fitted to a single exponential function (blue). **B** Time course for the hydrolysis of 5 μM substrate I by 0.5 nM PTE_H254ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue).

Substrate II

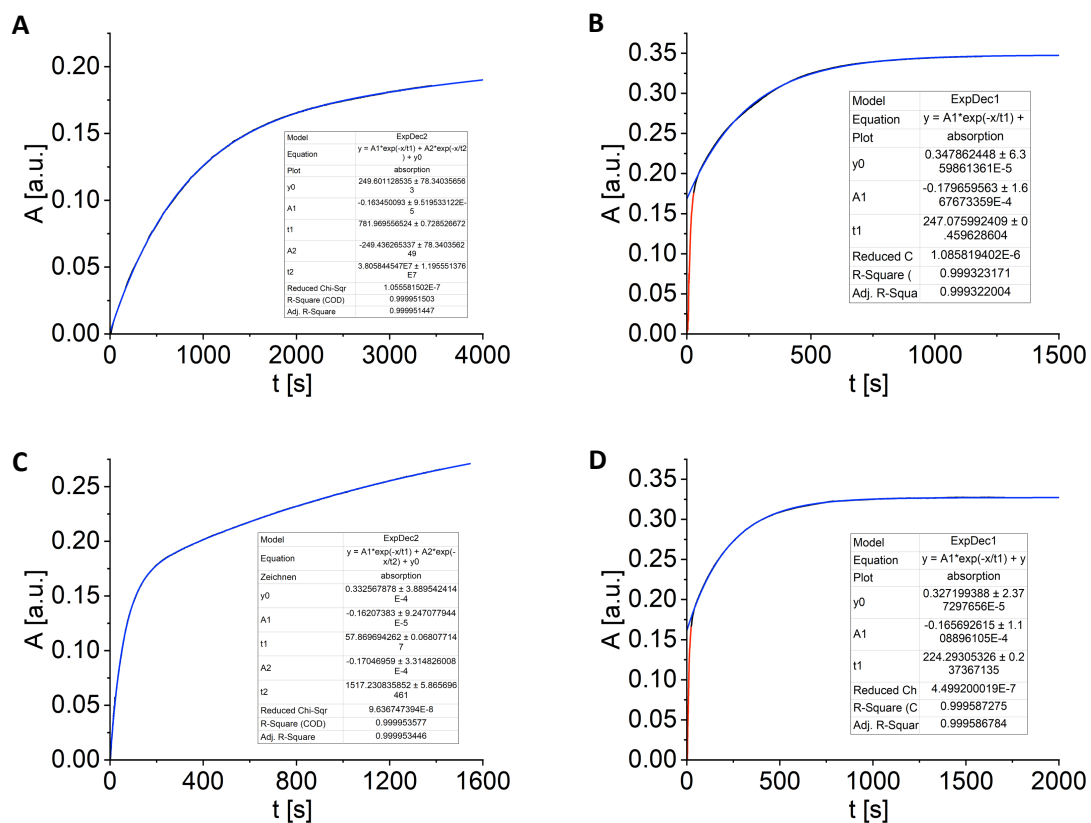


Figure S 13: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254ONBY before and after illumination

A Time course for the hydrolysis of 20 μM II-S_p/R_p by 2 nM PTE_H254ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of II-S_p. **B** Time course for the hydrolysis of 20 μM II-S_p/R_p by 20 nM PTE_H254ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of II-R_p. Data points corresponding to the hydrolysis of II-S_p (red) were not considered in the fit. **C** Time course for the hydrolysis of 20 μM II-R_p/S_p by 4 nM PTE_H254ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of II-S_p after irradiation at 365 nm. **D** Time course for the hydrolysis of 20 μM II-R_p/S_p by 20 nM PTE_H254ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of II-R_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of II-S_p (red) were not considered in the fit.

Substrate III

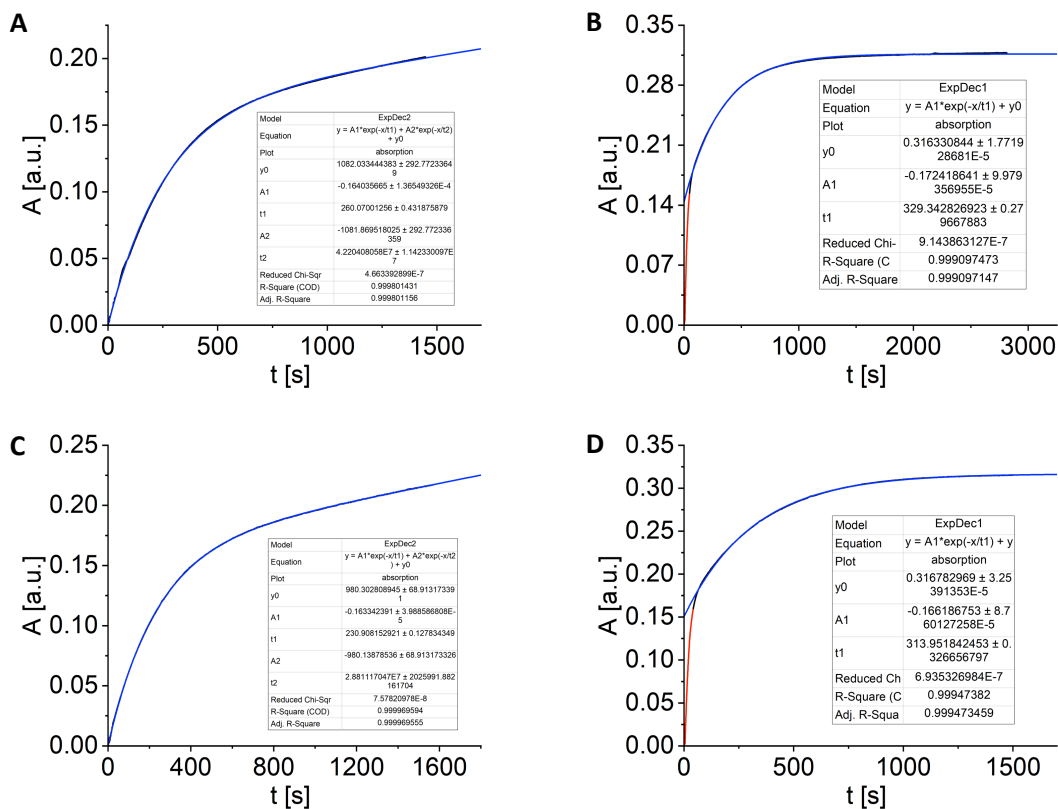


Figure S 14: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254ONBY before and after illumination

A Time course for the hydrolysis of 20 μM III-S_P/R_P by 2 nM PTE_H254ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of III-S_P. **B** Time course for the hydrolysis of 20 μM III-S_P/R_P by 15 nM PTE_H254ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of III-R_P. Data points corresponding to the hydrolysis of III-S_P (red) were not considered in the fit. **C** Time course for the hydrolysis of 20 μM III-R_P/S_P by 2 nM PTE_H254ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of III-S_P after irradiation at 365 nm. **D** Time course for the hydrolysis of 20 μM III-R_P/S_P by 15 nM PTE_H254ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of III-R_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of III-S_P (red) were not considered in the fit.

Substrate IV

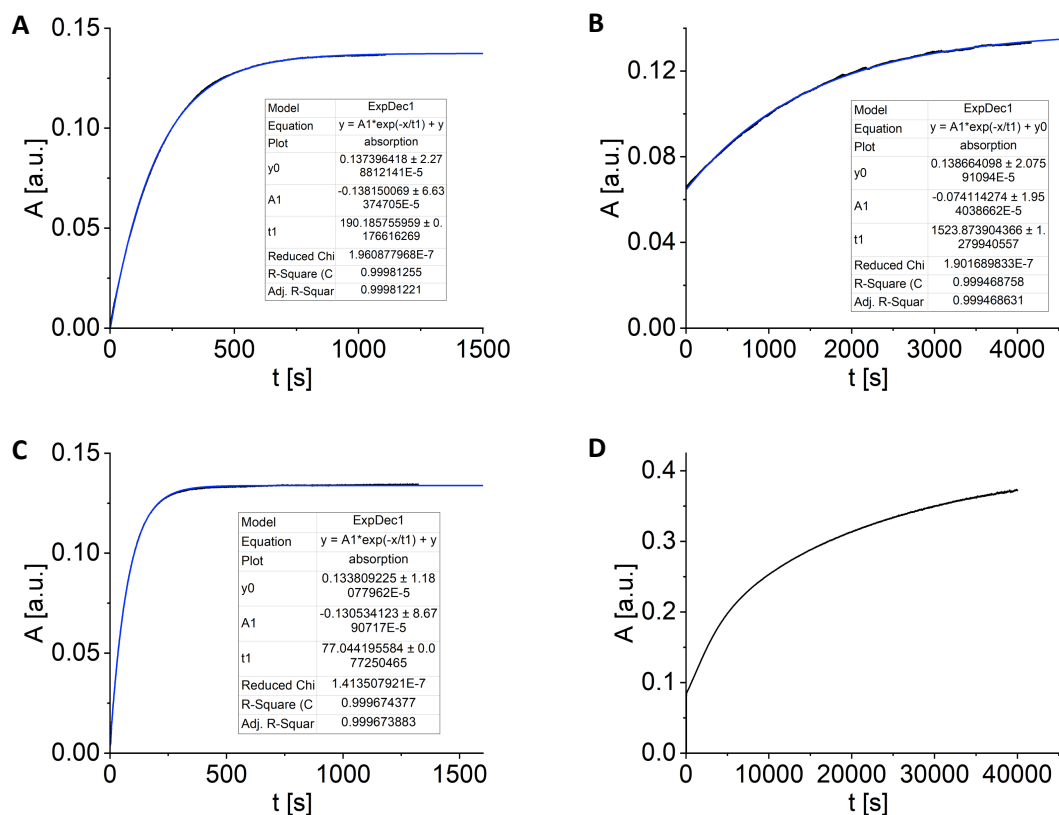


Figure S 15: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254ONBY before and after illumination

A Time course for the hydrolysis of 20 μM IV-S_p/R_p by 10 nM PTE_H254ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of IV-S_p. **B** Time course for the hydrolysis of 10 μM IV-S_p/R_p by 20 μM PTE_H254ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of IV-R_p. Data points corresponding to the hydrolysis of IV-S_p were not considered in the fit. **C** Time course for the hydrolysis of 20 μM IV-S_p/R_p by 20 nM PTE_H254ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of IV-S_p after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM IV-S_p/R_p by 20 μM PTE_H254ONBY after irradiation at 365 nm (black). The absorption exceeded the expected value for complete hydrolysis of 10 μM substrate IV and thus, was not fitted.

Substrate V

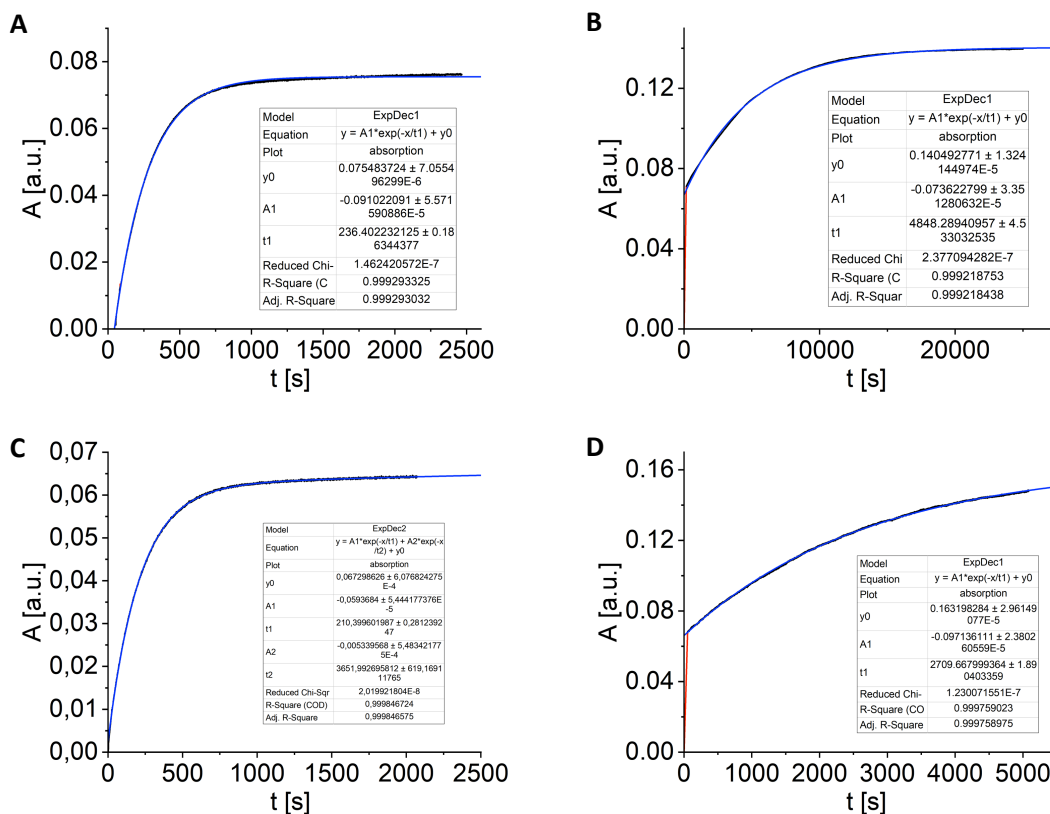


Figure S 16: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254ONBY before and after illumination

A Time course for the hydrolysis of $5 \mu\text{M}$ V-S_p/R_p by 10 nM PTE_H254ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of V-S_p. **B** Time course for the hydrolysis of $10 \mu\text{M}$ V-S_p/R_p by $5 \mu\text{M}$ PTE_H254ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of V-R_p. Data points corresponding to the hydrolysis of V-S_p (red) were not considered in the fit. **C** Time course for the hydrolysis of $10 \mu\text{M}$ V-S_p/R_p by 5 nM PTE_H254ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of V-S_p after irradiation at 365 nm . **D** Time course for the hydrolysis of $10 \mu\text{M}$ V-S_p/R_p by $15 \mu\text{M}$ PTE_H254ONBY after irradiation at 365 nm . The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254ONBY for the hydrolysis of V-R_p after irradiation at 365 nm . Data points corresponding to the hydrolysis of V-S_p (red) were not considered in the fit.

1.1.5 PTE_H254Y #1

Substrate I

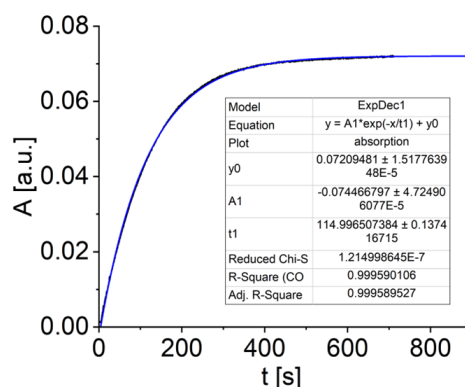


Figure S 17: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254Y #1

Time course for the hydrolysis of 5 μM substrate I by addition of 5 nM PTE_H254Y #1 (black). The data was fitted to a single exponential function.

Substrate II

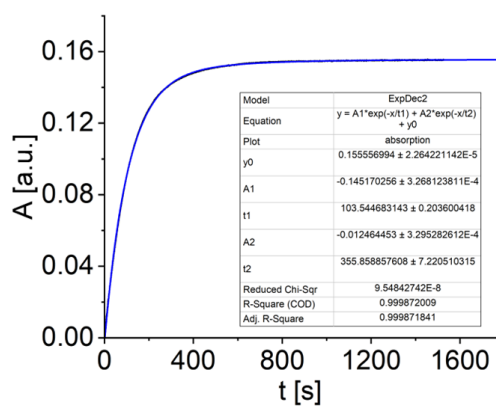


Figure S 18: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254Y #1

A Time course for the hydrolysis of 10 μM II-S_p/R_p by 200 nM PTE_H254Y #1 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #1 for the hydrolysis of II-S_p/R_p.

Substrate III

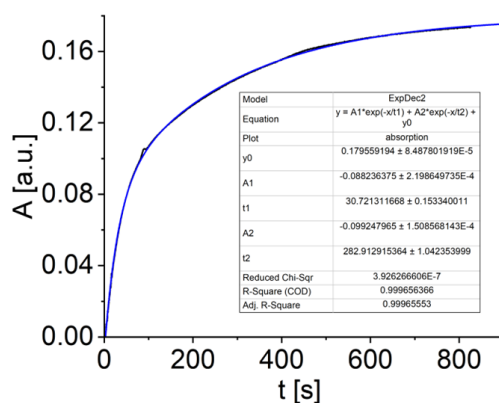


Figure S 19: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254Y #1

A Time course for the hydrolysis of $10 \mu\text{M}$ III-S_P/R_P by 200 nM PTE_H254Y #1 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #1 for the hydrolysis of III-S_P/III-R_P.

Substrate IV

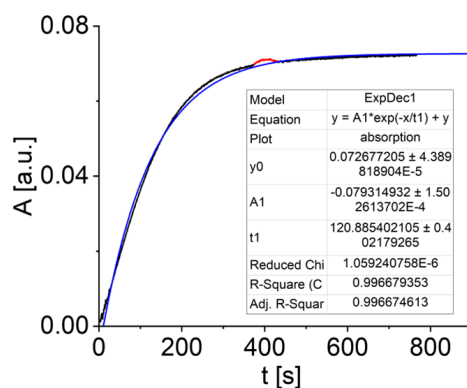


Figure S 20: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254Y #1

A Time course for the hydrolysis of $10 \mu\text{M}$ IV-S_P/R_P by 500 nM PTE_H254Y #1 (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #1 for the hydrolysis of IV-S_P.

Substrate V

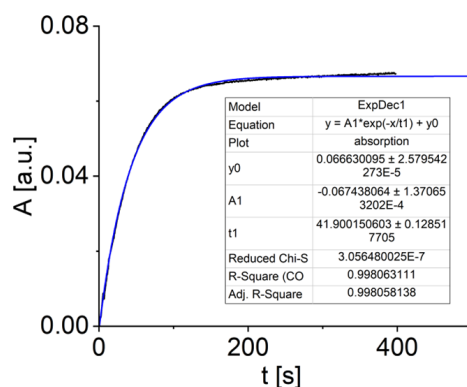


Figure S 21: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254Y #1

A Time course for the hydrolysis of 10 μM V-S_p/R_p by 50 nM PTE_H254Y #1 (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #1 for the hydrolysis of V-S_p.

1.1.6 PTE_H254Y #2

Substrate I

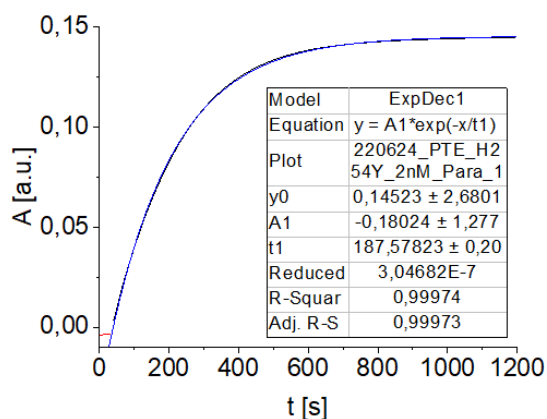


Figure S 22: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254Y #2

Time course for the hydrolysis of 10 μM substrate I by addition of 2 nM PTE_H254Y #2 (black). The data was fitted to a single exponential function.

Substrate II

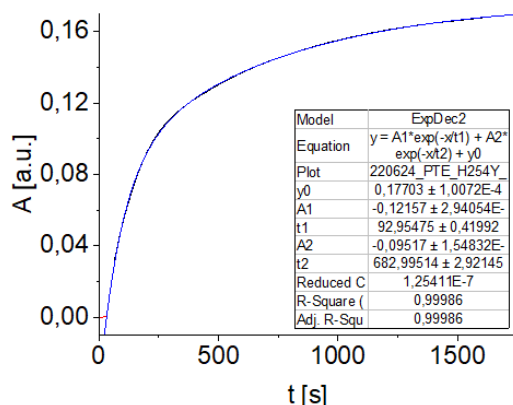


Figure S 23: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254Y #2

A Time course for the hydrolysis of $10 \mu\text{M}$ II-S_P/R_P by 50 nM PTE_H254Y #2 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #2 for the hydrolysis of II-S_P/R_P.

Substrate III

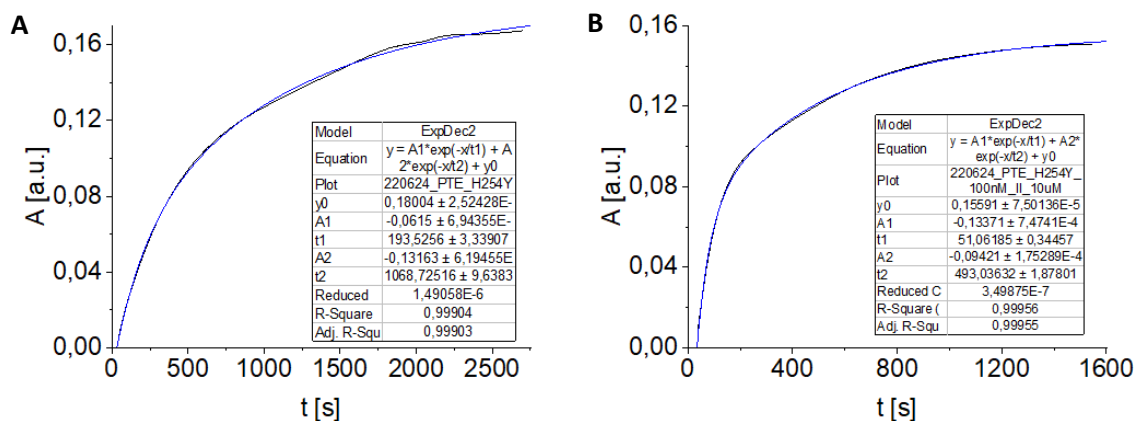


Figure S 24: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254Y #2

A Time course for the hydrolysis of $10 \mu\text{M}$ III-S_P/R_P by 10 nM PTE_H254Y #2 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #2 for the hydrolysis of III-S_P. **B** Time course for the hydrolysis of $10 \mu\text{M}$ III-S_P/R_P by 100 nM PTE_H254Y #2 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #2 for the hydrolysis of III-R_P.

Substrate IV

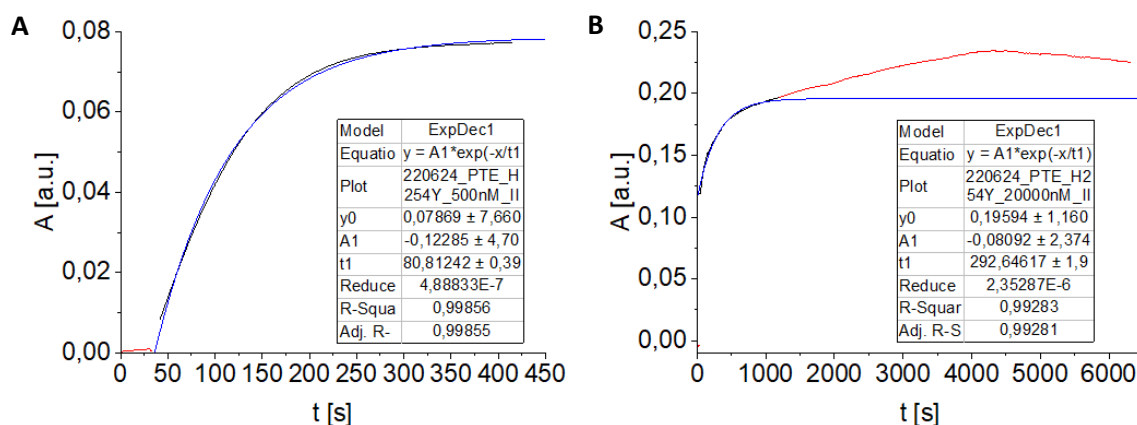


Figure S 25: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254Y #2

A Time course for the hydrolysis of 10 μM IV-S_p/R_p by 500 nM PTE_H254Y #2 (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #2 for the hydrolysis of IV-S_p. **B** Time course for the hydrolysis of 10 μM IV-S_p/R_p by 20 μM PTE_H254Y #2 (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #2 for the hydrolysis of IV-R_p. Data points corresponding to the hydrolysis of IV-S_p were not considered in the fit.

Substrate V

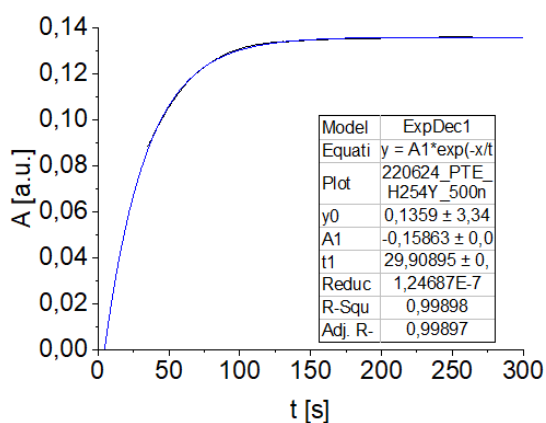


Figure S 26: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254Y #2

A Time course for the hydrolysis of 10 μM V-S_p/R_p by 500 nM PTE_H254Y #2 (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #2 for the hydrolysis of V-S_p/R_p.

1.1.7 PTE_H254Y #3

Substrate I

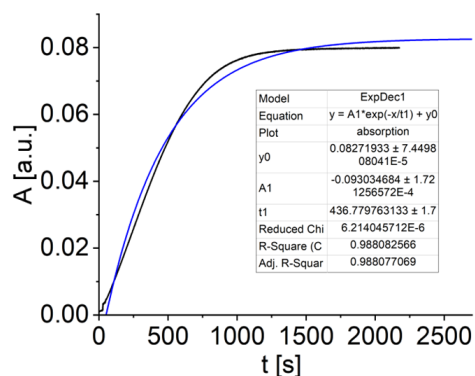


Figure S 27: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254Y #3

Time course for the hydrolysis of 5 μM substrate I by addition of 50 nM PTE_H254Y #3 (black). The data was fitted to a single exponential function.

Substrate II

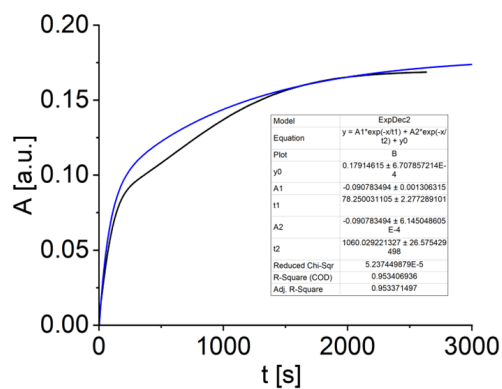


Figure S 28: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254Y #3

A Time course for the hydrolysis of 10 μM II-S_P/R_P by 200 nM PTE_H254Y #3 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #3 for the hydrolysis of II-S_P/R_P.

Substrate III

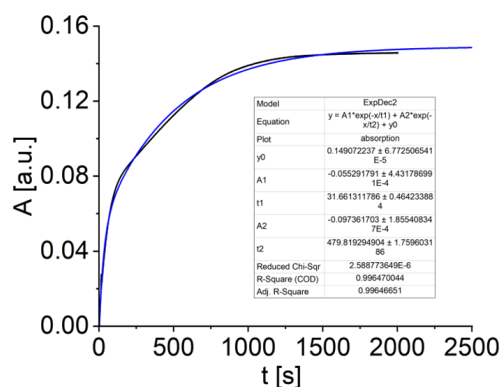


Figure S 29: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254Y #3

A Time course for the hydrolysis of 10 μM III-S_P/R_P by 200 nM PTE_H254Y #3 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #3 for the hydrolysis of III-S_P/R_P.

Substrate IV

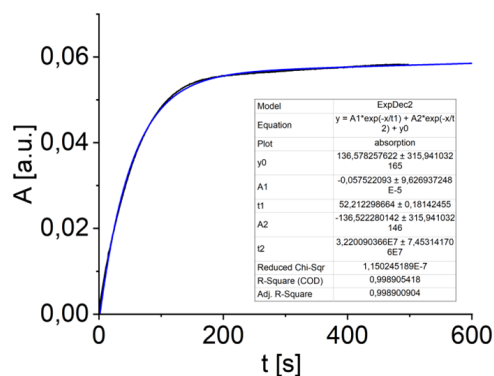


Figure S 30: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254Y #3

A Time course for the hydrolysis of 10 μM IV-S_P/R_P by 1 μM PTE_H254Y #3 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #3 for the hydrolysis of IV-S_P.

Substrate V

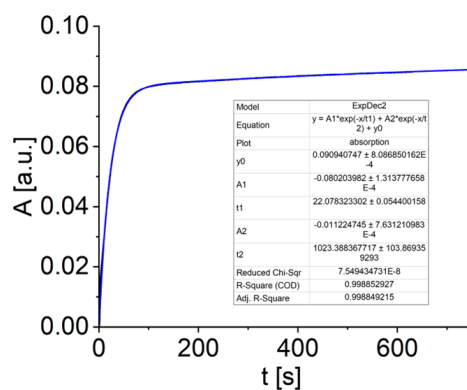


Figure S 31: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254Y #3

A Time course for the hydrolysis of 10 μM V-S_P/R_P by 1 μM PTE_H254Y #3 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #3 for the hydrolysis of V-S_P.

1.1.8 PTE_H257ONBY

Substrate I

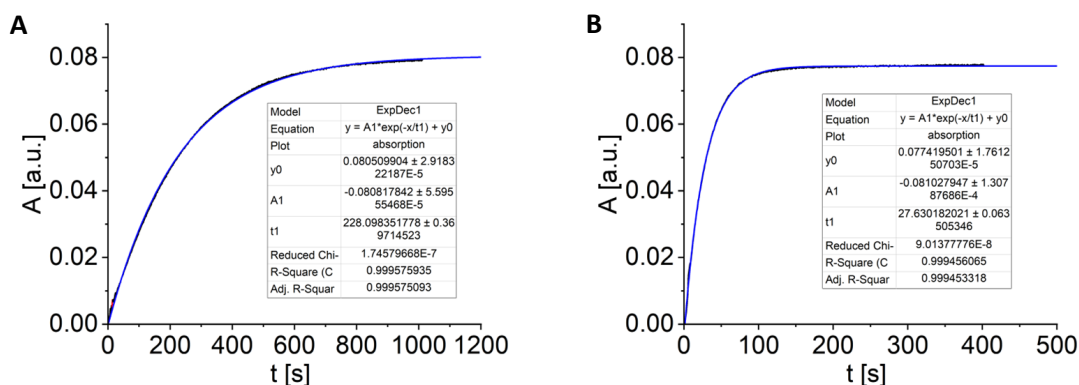


Figure S 32: Enzymatic hydrolysis of substrate I catalyzed by PTE_H257ONBY before and after illumination

A Time course for the hydrolysis of 5 μM substrate I by 1 nM PTE_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue). **B** Time course for the hydrolysis of 5 μM substrate I by 1 nM PTE_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue).

Substrate II

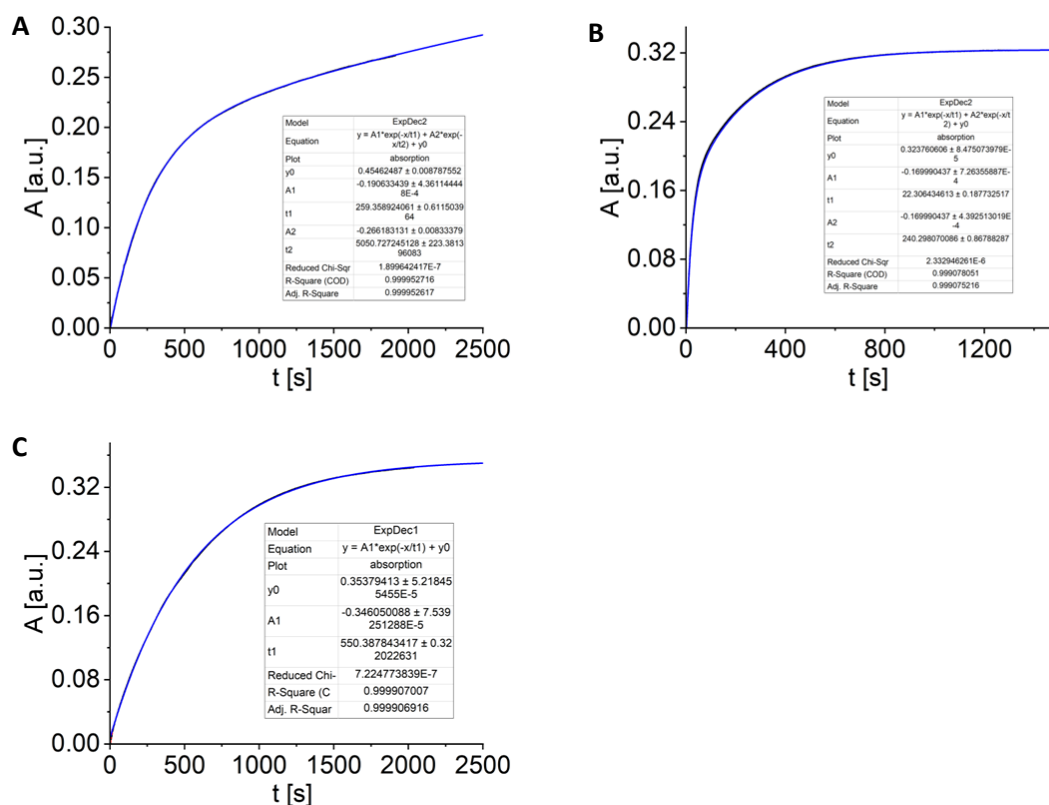


Figure S 33: Progress curve for PTE_H257ONBY catalyzed substrate II hydrolysis

A Time course for the hydrolysis of 20 μM II-S_P/R_P by 2 nM PTE_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of II-S_P. **B** Time course for the hydrolysis of 20 μM II-R_P/S_P by 15 nM PTE_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY *as isolated* for the hydrolysis of II-R_P. **C** Time course for the hydrolysis of 20 μM II-R_P/S_P by 1 nM PTE_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of II-S_P/R_P after irradiation at 365 nm.

Substrate III

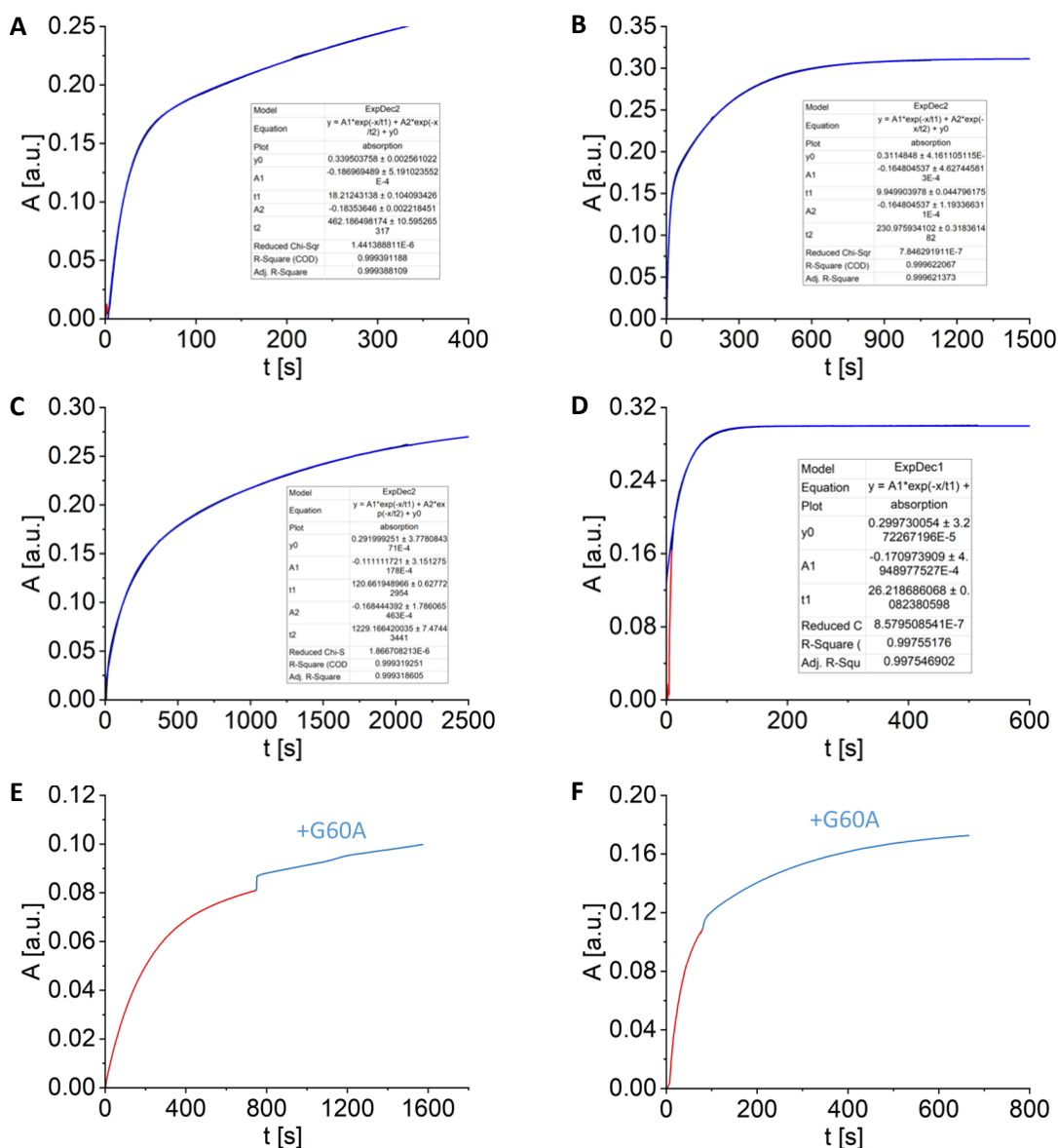


Figure S 34: Enzymatic hydrolysis of substrate III catalyzed by PTE_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM III- S_P / R_P by 2 nM PTE_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of III- S_P . **B** Time course for the hydrolysis of 10 μM III- S_P / R_P by 20 nM PTE_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of III- R_P . **C** Time course for the hydrolysis of 10 μM III- R_P / S_P by 1 nM PTE_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of III- S_P after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM III- R_P / S_P by 15 nM PTE_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of III- R_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of III- S_P (red) were not considered in the fit. **E** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_H257ONBY *as isolated*. Enzymatic hydrolysis of 10 μM substrate III was initiated by addition of 1 nM PTE_H257ONBY and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that III- S_P is preferentially hydrolyzed. **F** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μM substrate III was initiated by addition of 1 nM PTE_H257ONBY and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected upon addition of PTE_G60A, implying that III- S_P is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

Substrate IV

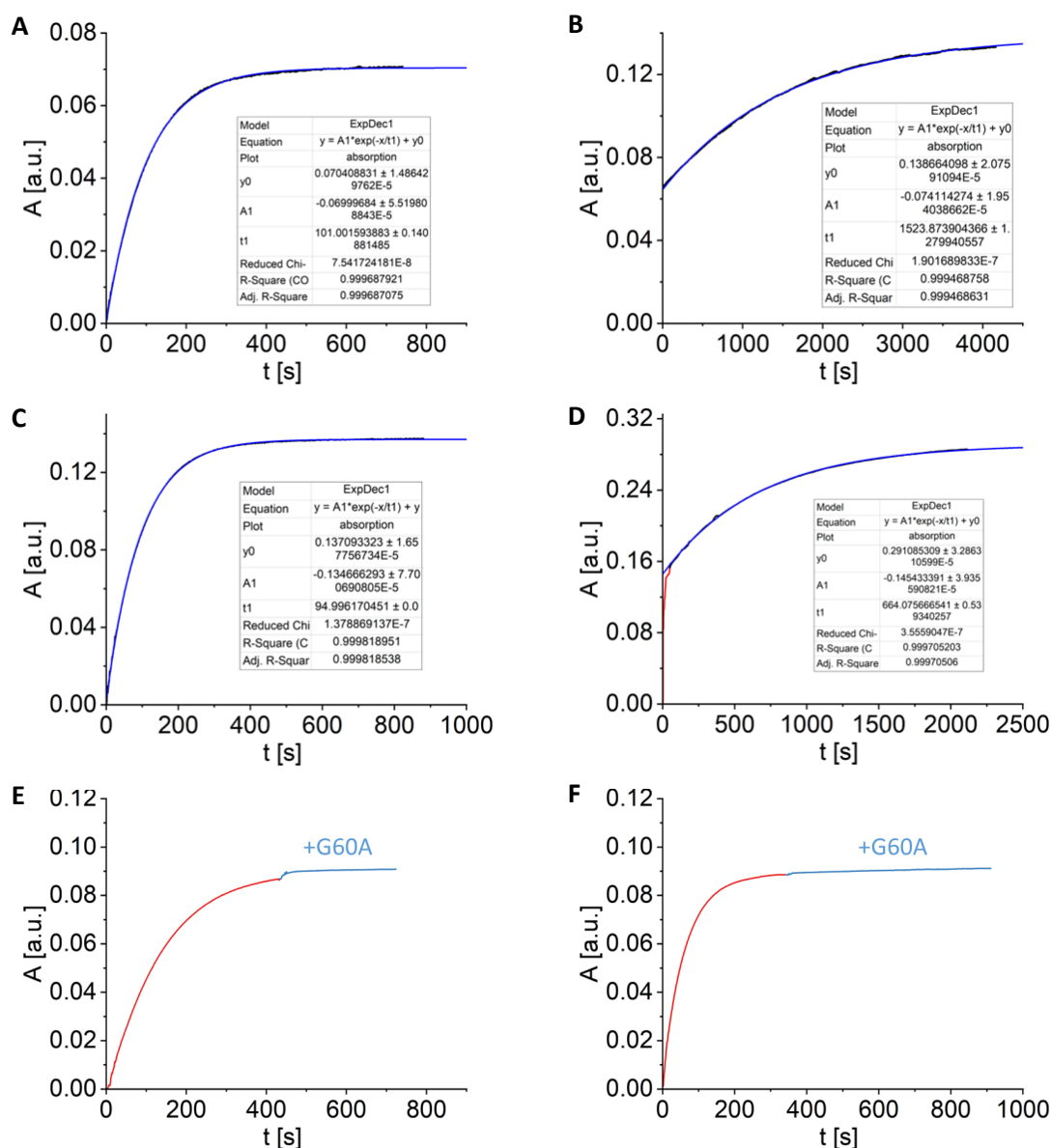


Figure S 35: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM IV- S_p/R_p by 10 nM PTE_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of IV- S_p . **B** Time course for the hydrolysis of 10 μM IV- S_p/R_p by 20 μM PTE_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of IV- R_p . Data points corresponding to the hydrolysis of IV- S_p (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM IV- S_p/R_p by 10 nM PTE_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of IV- S_p after irradiation at 365 nm. **D** Time course for the hydrolysis of 20 μM IV- S_p/R_p by 3 μM PTE_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of IV- R_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of IV- S_p (red) were not considered in the fit. **E** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_H257ONBY *as isolated*. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 10 nM PTE_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that IV- S_p is preferentially hydrolyzed. **F** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 10 nM PTE_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected upon addition of PTE_G60A, implying that IV- S_p is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

Substrate V

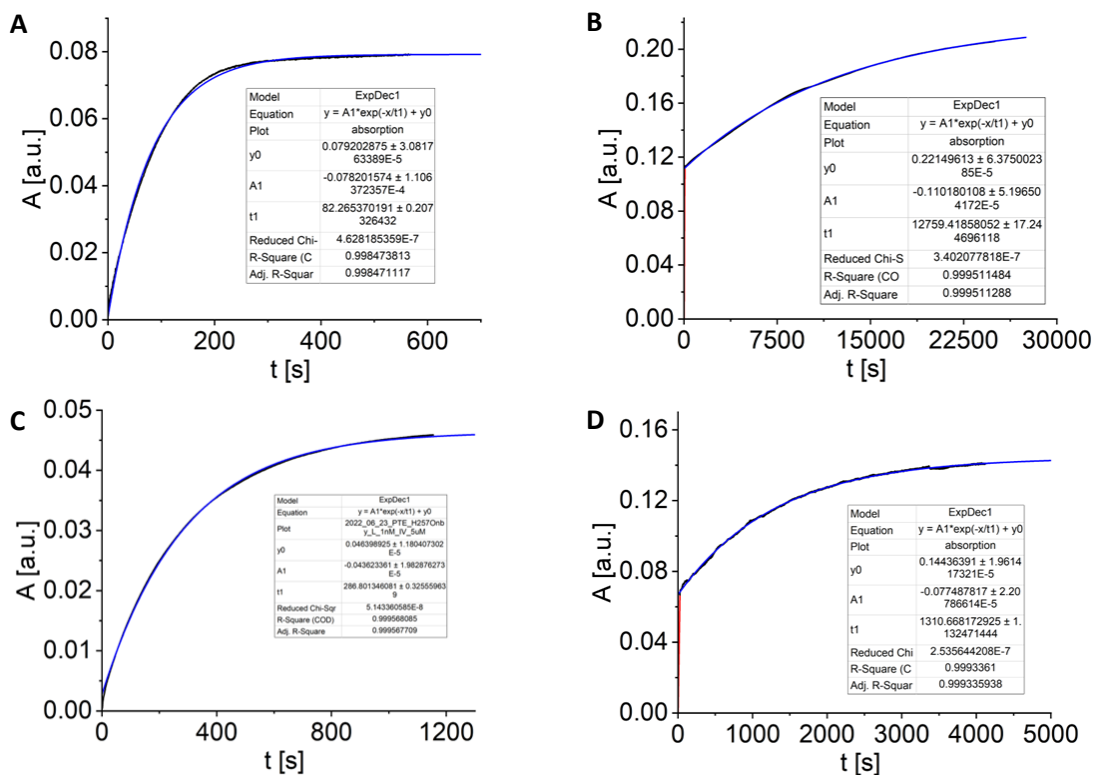


Figure S 36: Enzymatic hydrolysis of substrate V catalyzed by PTE_H257ONBY before and after illumination

A Time course for the hydrolysis of $10 \mu\text{M}$ V-S_P/R_P by 10 nM PTE_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of V-S_P. **B** Time course for the hydrolysis of $10 \mu\text{M}$ V-S_P/R_P by $3.6 \mu\text{M}$ PTE_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of V-R_P. Data points corresponding to the hydrolysis of V-S_P (red) were not considered in the fit. **C** Time course for the hydrolysis of $5 \mu\text{M}$ V-S_P/R_P by 1 nM PTE_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of V-S_P after irradiation at 365 nm . **D** Time course for the hydrolysis of $10 \mu\text{M}$ V-S_P/R_P by $10 \mu\text{M}$ PTE_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257ONBY for the hydrolysis of V-R_P after irradiation at 365 nm . Data points corresponding to the hydrolysis of V-S_P (red) were not considered in the fit.

1.1.9 PTE_H257Y

Substrate I

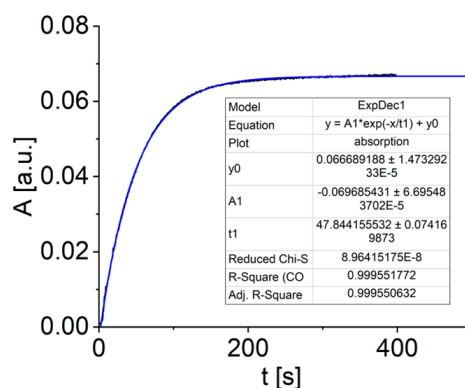


Figure S 37: Enzymatic hydrolysis of substrate I catalyzed by PTE_H257Y

Time course for the hydrolysis of 5 μ M substrate I by addition of 0.5 nM PTE_H257Y (black). The data was fitted to a single exponential function.

Substrate II

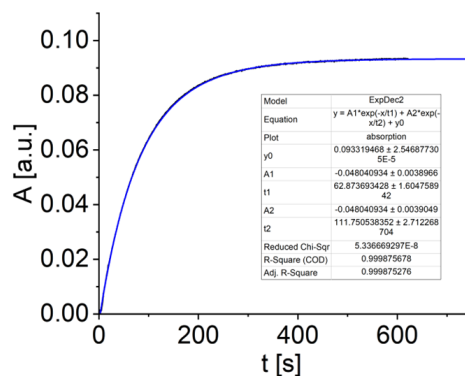


Figure S 38: Enzymatic hydrolysis of substrate II catalyzed by PTE_H257Y

A Time course for the hydrolysis of 5 μ M II-S_P/R_P by 3 nM PTE_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257Y for the hydrolysis of II-S_P/R_P.

Substrate III

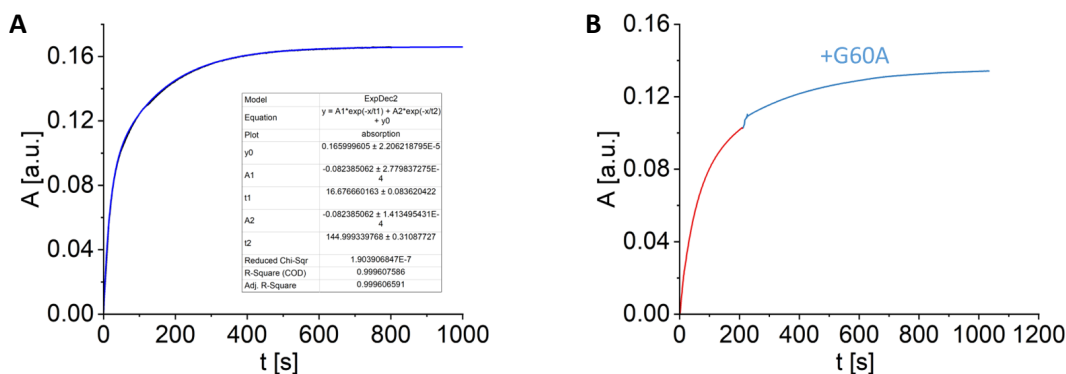


Figure S 39: Enzymatic hydrolysis of substrate III catalyzed by PTE_H257Y

A Time course for the hydrolysis of 10 μM III-S_P/R_P by 2 nM PTE_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257Y for the hydrolysis of III-S_P/R_P. **B** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_H257Y. Enzymatic hydrolysis of 10 μM substrate III was initiated by addition of 1 nM PTE_H257Y and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that III-S_P is preferentially hydrolyzed.

Substrate IV

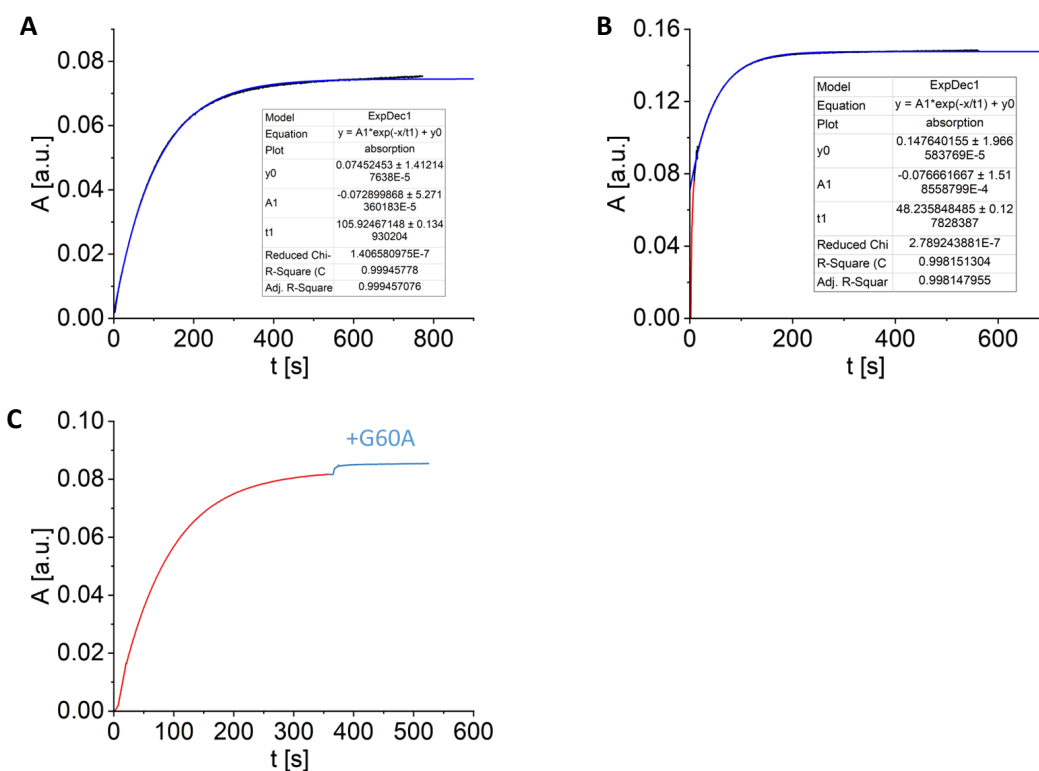


Figure S 40: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H257Y

A Time course for the hydrolysis of 10 μM IV-S_P/R_P by 10 nM PTE_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257Y for the hydrolysis of IV-S_P. **B** Time course for the hydrolysis of 10 μM IV-S_P/R_P by 5 μM PTE_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257Y for the hydrolysis of IV-R_P. Data points corresponding to the hydrolysis of IV-S_P (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_H257Y. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 50 nM PTE_H257Y and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that IV-S_P is preferentially hydrolyzed.

Substrate V

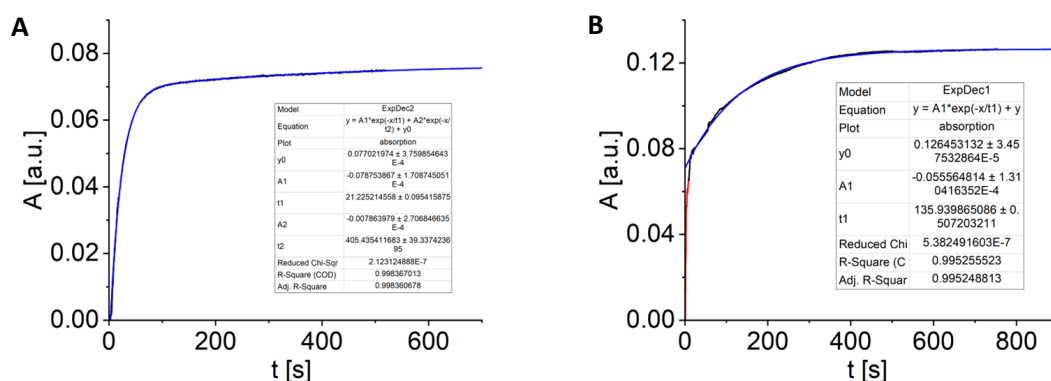


Figure S 41: Enzymatic hydrolysis of substrate V catalyzed by PTE_H257Y

A Time course for the hydrolysis of $10 \mu\text{M}$ V-S_P/R_P by 5 nM PTE_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257Y for the hydrolysis of V-S_P. **B** Time course for the hydrolysis of $10 \mu\text{M}$ V-S_P/R_P by 25 μM PTE_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H257Y for the hydrolysis of V-R_P. Data points corresponding to the hydrolysis of V-S_P (red) were not considered in the fit.

1.1.10 PTE_I106A_H257ONBY

Substrate I

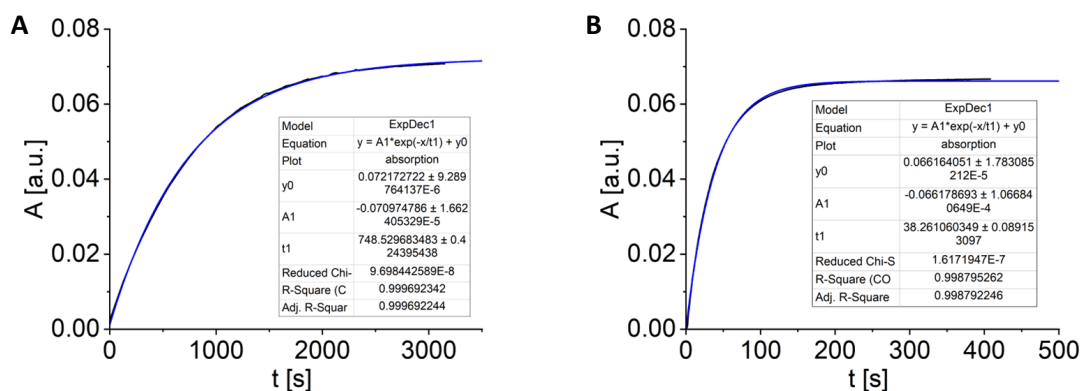


Figure S 42: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_H257ONBY before and after illumination

A Time course for the hydrolysis of $5 \mu\text{M}$ substrate I by 2 nM PTE_I106A_H257ONBY as isolated (black). The data was fitted to a single exponential function (blue). **B** Time course for the hydrolysis of $5 \mu\text{M}$ substrate I by 2 nM PTE_I106A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue).

Substrate II

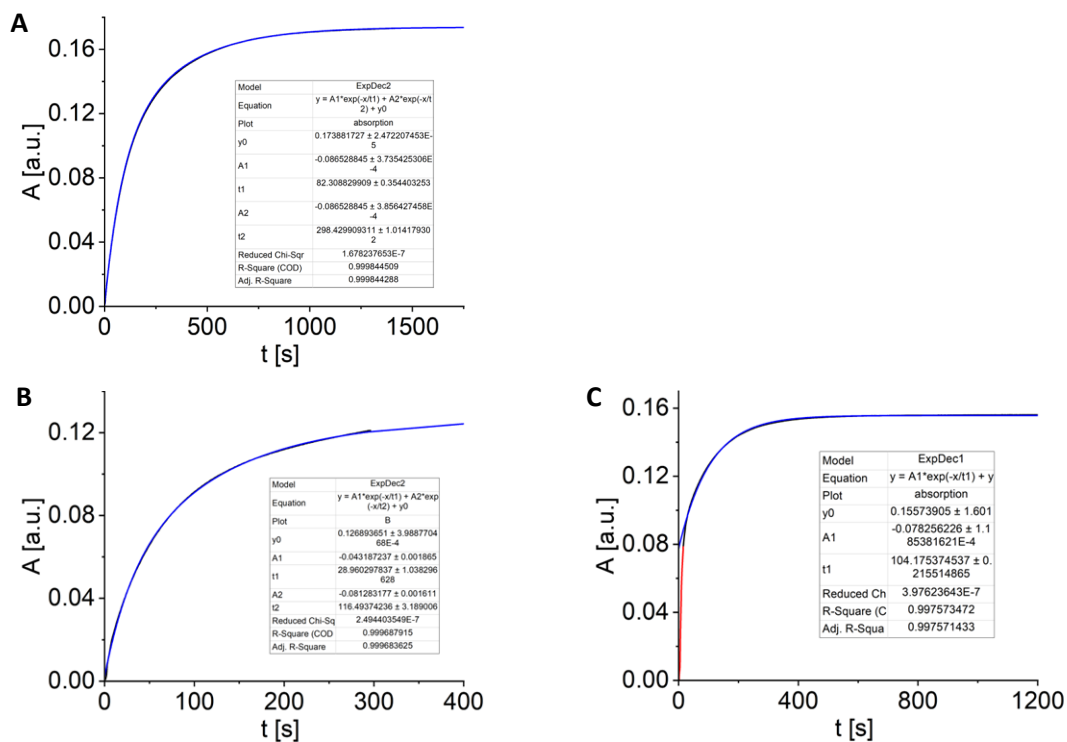


Figure S 43: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μ M II-S_P/R_P by 10 nM PTE_I106A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of II-S_P/R_P. **B** Time course for the hydrolysis of 10 μ M II-R_P/S_P by 0.5 nM PTE_I106A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of II-R_P after irradiation at 365 nm. **C** Time course for the hydrolysis of 10 μ M II-R_P/S_P by 5 nM PTE_I106A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of II-S_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of II-R_P (red) were not considered in the fit.

Substrate III

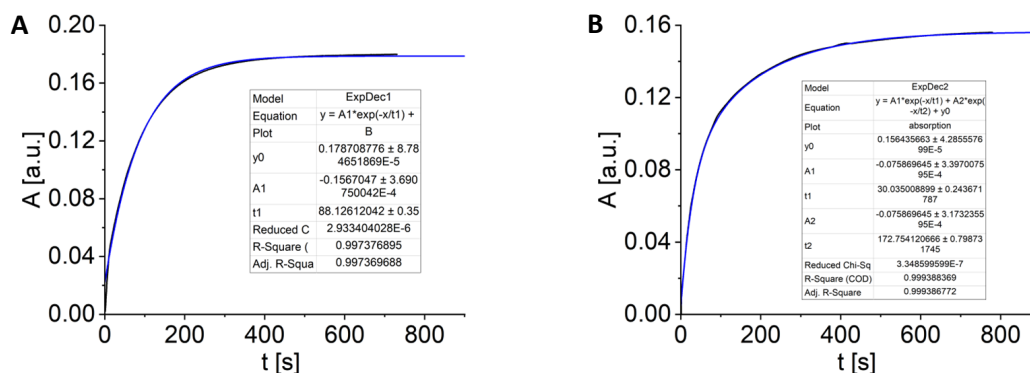


Figure S 44: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μ M III-S_P/R_P by 5 nM PTE_I106A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of III-S_P/R_P. **B** Time course for the hydrolysis of 10 μ M III-R_P/S_P by 2 nM PTE_I106A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of III-R_P/S_P after irradiation at 365 nm.

Substrate IV

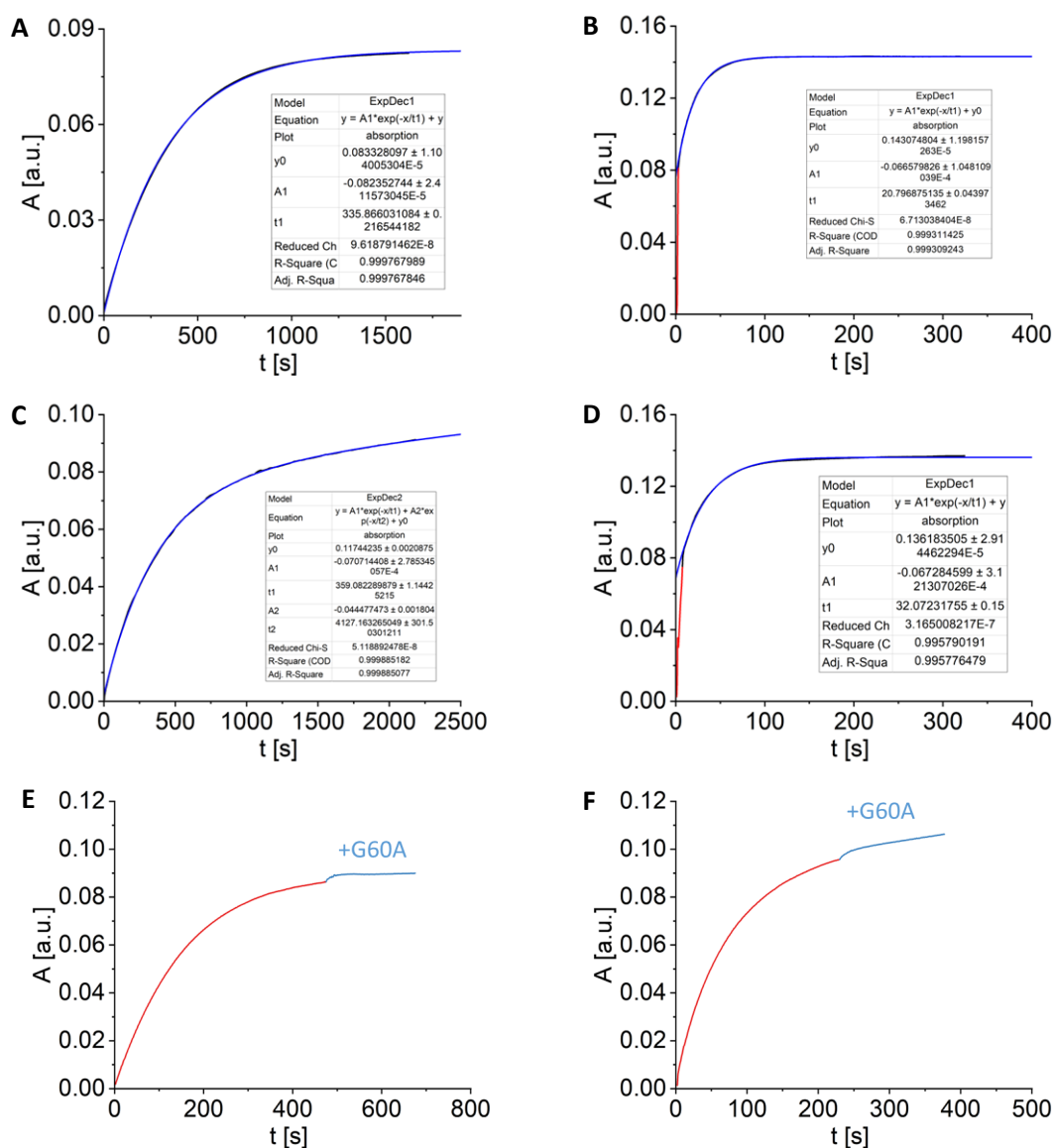


Figure S 45: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM IV- S_p/R_p by 20 nM PTE_I106A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of IV- S_p . **B** Time course for the hydrolysis of 10 μM IV- S_p/R_p by 1 μM PTE_I106A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of IV- R_p . Data points corresponding to the hydrolysis of IV- S_p (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM IV- S_p/R_p by 10 nM PTE_I106A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of IV- S_p after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM IV- S_p/R_p by 1 μM PTE_I106A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of IV- R_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of IV- S_p (red) were not considered in the fit. **E** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H257ONBY *as isolated*. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 30 nM PTE_I106A_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that IV- S_p is preferentially hydrolyzed. **F** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 30 nM PTE_I106A_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected upon addition of PTE_G60A, implying that IV- S_p is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

Substrate V

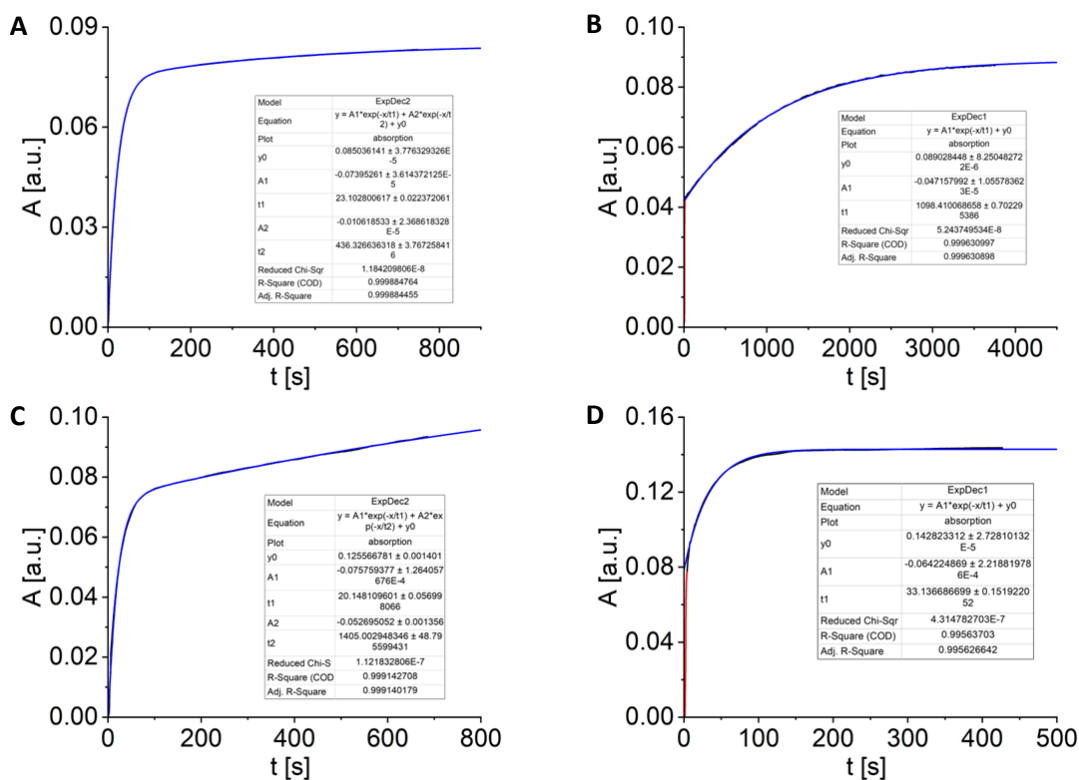


Figure S 46: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM V-S_p/R_p by 40 nM PTE_I106A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of V-S_p. **B** Time course for the hydrolysis of 5 μM V-S_p/R_p by 1 μM PTE_I106A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of V-R_p. Data points corresponding to the hydrolysis of V-S_p (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM V-S_p/R_p by 40 nM PTE_I106A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of V-S_p after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM V-S_p/R_p by 1.5 μM PTE_I106A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of V-R_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of V-S_p (red) were not considered in the fit.

1.1.11 PTE_I106A_H257Y

Substrate I

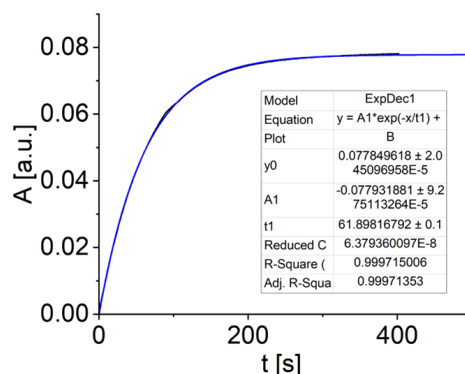


Figure S 47: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_H257Y

Time course for the hydrolysis of 5 μM substrate I by addition of 2 nM PTE_I106A_H257Y (black). The data was fitted to a single exponential function.

Substrate II

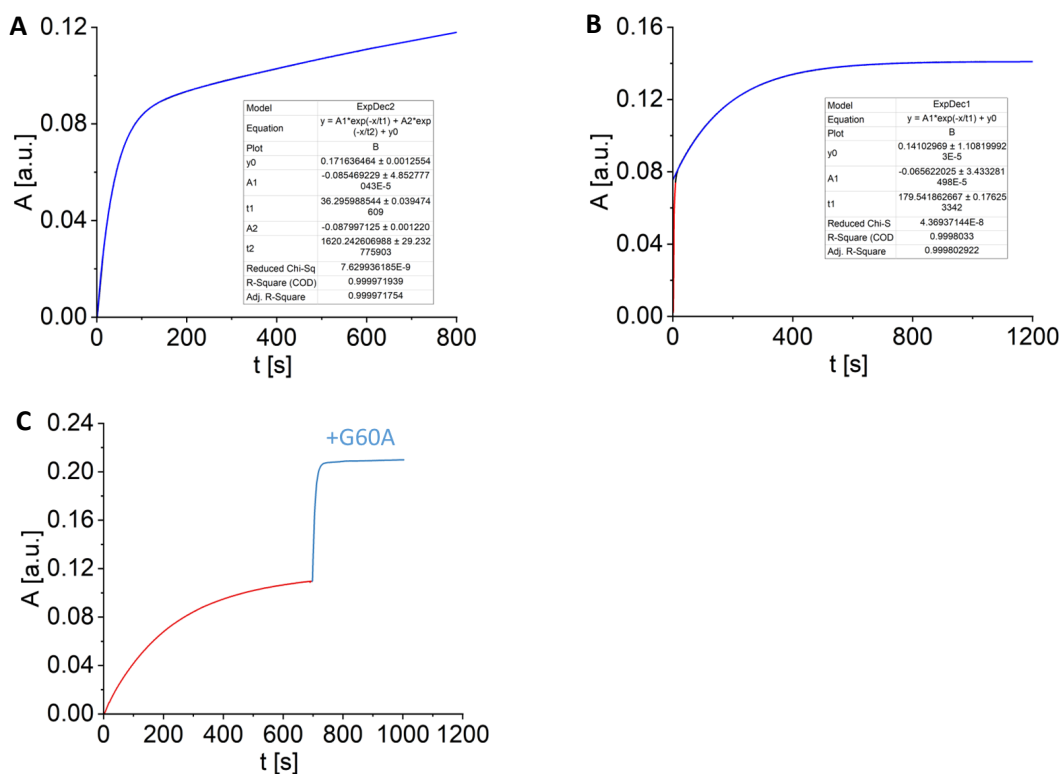


Figure S 48: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_H257Y

A Time course for the hydrolysis of 10 μM II- R_p/S_p by 0.5 nM PTE_I106A_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for the hydrolysis of II- R_p . **B** Time course for the hydrolysis of 10 μM II- R_p/S_p by 5 nM PTE_I106A_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for the hydrolysis of II- S_p . Data points corresponding to the hydrolysis of II- R_p (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H257Y. Enzymatic hydrolysis of 10 μM substrate II was initiated by addition of 0.3 nM PTE_I106A_H257Y and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorbance upon addition of PTE_G60A indicates that II- R_p is preferentially hydrolyzed.

Substrate III

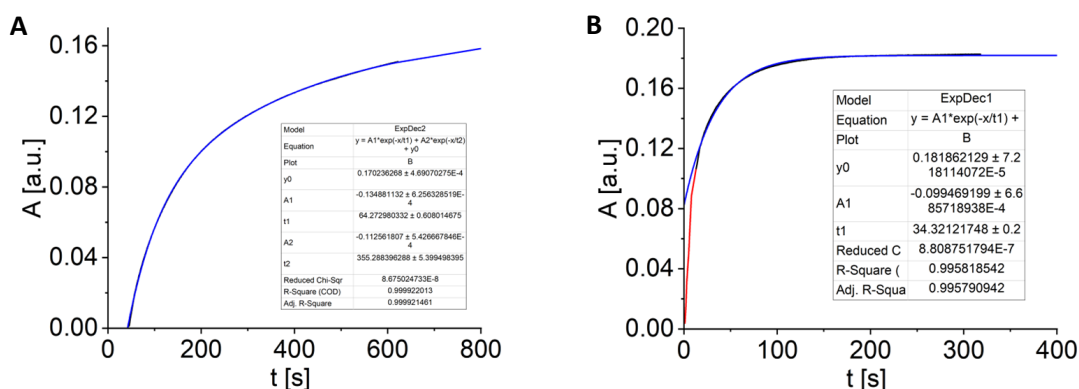


Figure S 49: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_H257Y

A Time course for the hydrolysis of 10 μM III-S_p/R_p by 0.5 nM PTE_I106A_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for the hydrolysis of III-R_p. **B** Time course for the hydrolysis of 10 μM III-R_p/S_p by 5 nM PTE_I106A_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for the hydrolysis of III-S_p. Data points corresponding to the hydrolysis of III-R_p (red) were not considered in the fit.

Substrate IV

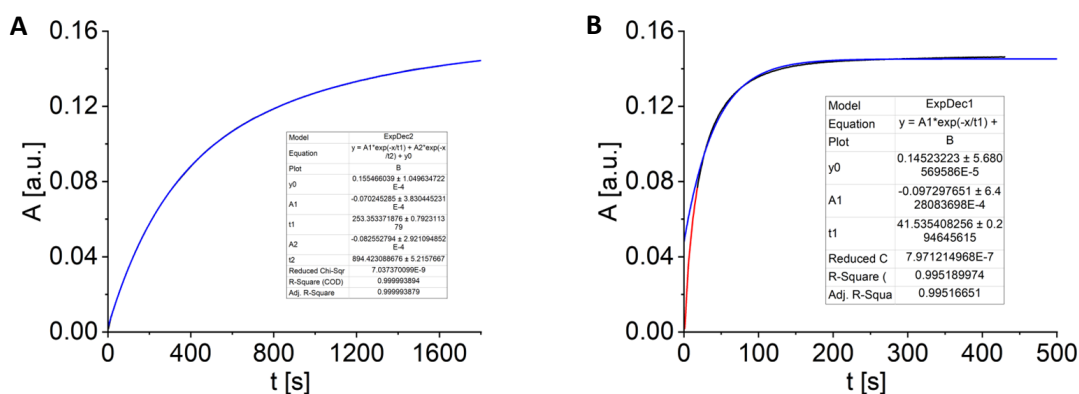


Figure S 50: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_H257Y

A Time course for the hydrolysis of 10 μM IV-S_p/R_p by 30 nM PTE_I106A_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for the hydrolysis of IV-S_p. **B** Time course for the hydrolysis of 10 μM IV-S_p/R_p by 500 nM PTE_I106A_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for the hydrolysis of IV-R_p. Data points corresponding to the hydrolysis of IV-S_p (red) were not considered in the fit.

Substrate V

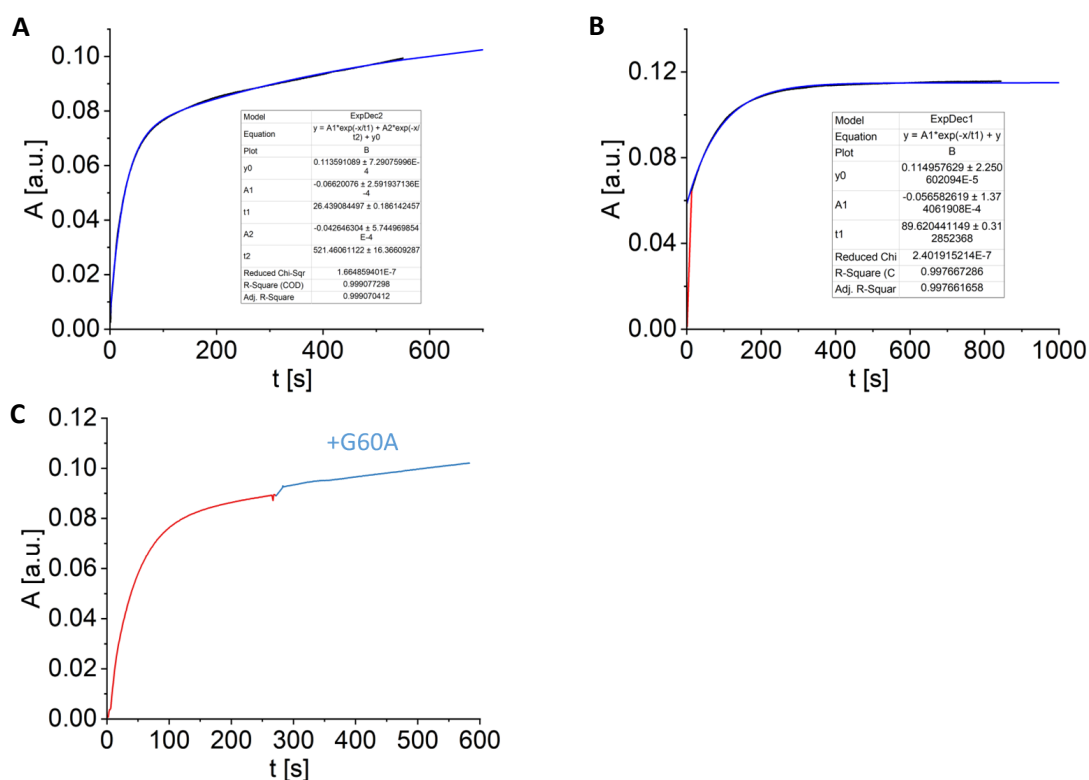


Figure S 51: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_H257Y

A Time course for the hydrolysis of 10 μM V-S_p/R_p by 30 nM PTE_I106A_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for the hydrolysis of V-S_p. **B** Time course for the hydrolysis of 10 μM V-S_p/R_p by 500 nM PTE_I106A_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for the hydrolysis of V-R_p. Data points corresponding to the hydrolysis of V-S_p (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H257Y. Enzymatic hydrolysis of 10 μM substrate V was initiated by addition of 30 nM PTE_I106A_H257Y and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that V-S_p is preferentially hydrolyzed.

1.1.12 PTE_I106A_F132A_H257ONBY

Substrate I

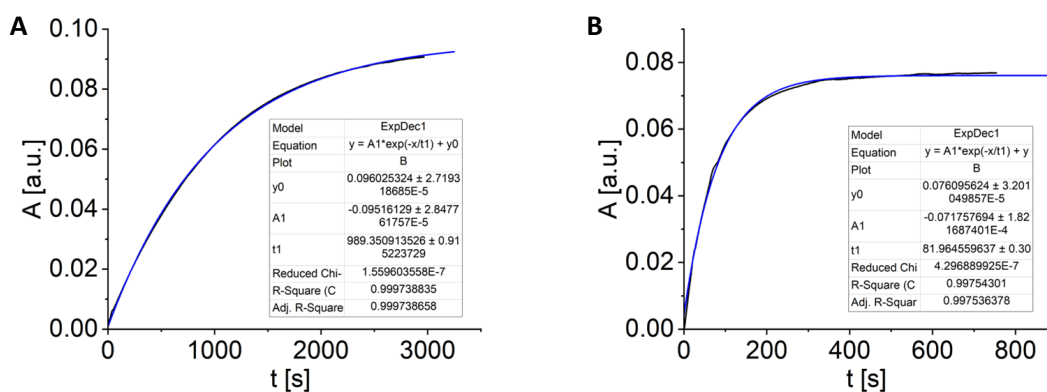


Figure S 52: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_F132A_H257ONBY before and after illumination

A Time course for the hydrolysis of 5 μ M substrate I by 5 nM PTE_I106A_F132A_H257ONBY *as isolated* (black). The data was fitted to a single exponential fit (blue). **B** Time course for the hydrolysis of 5 μ M substrate I by 1 nM PTE_I106A_F132A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential fit (blue).

Substrate II

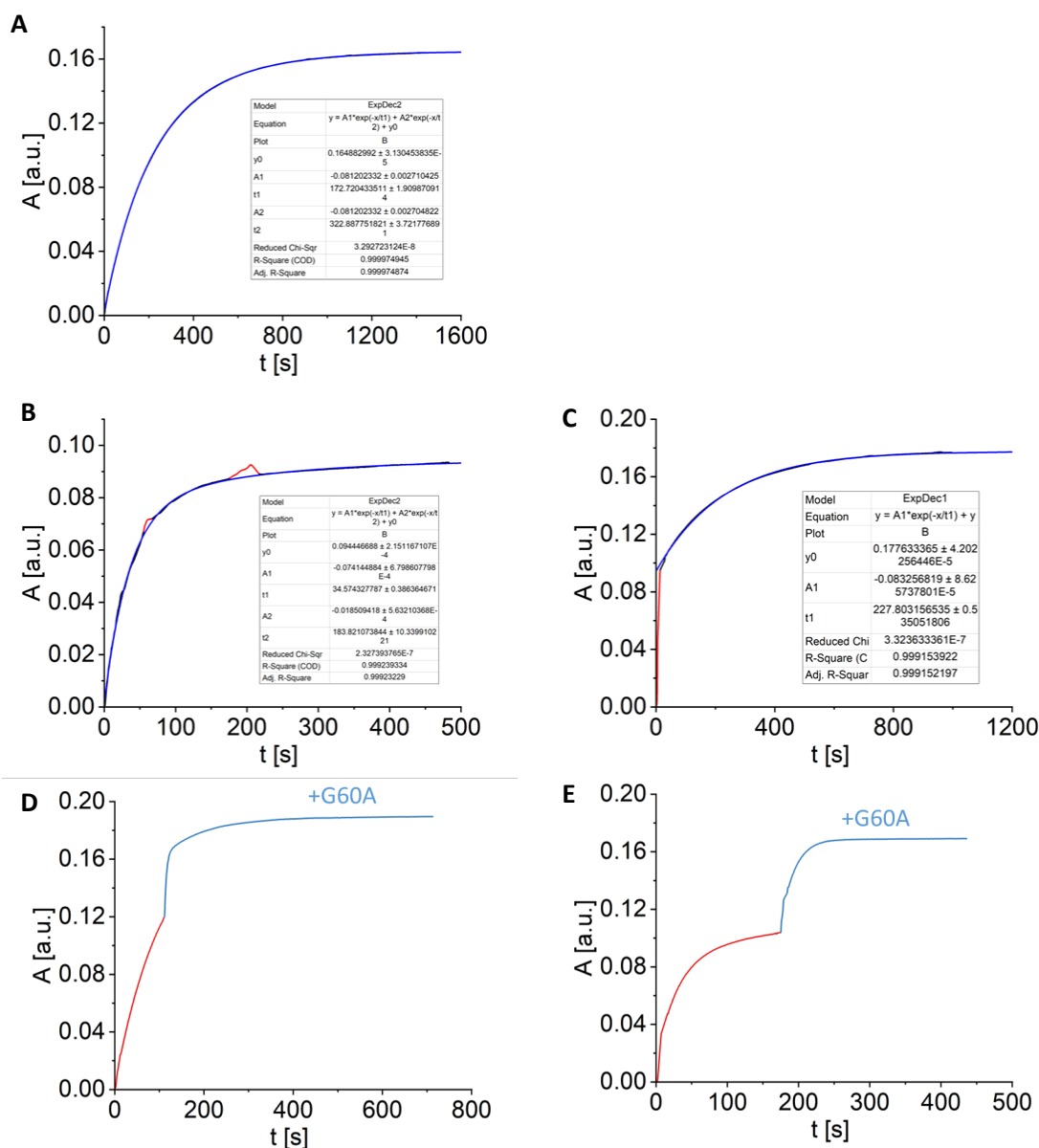


Figure S 53: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F132A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μ M II-S_p/R_p by 15 nM PTE_I106A_F132A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of II-S_p/R_p. **B** Time course for the hydrolysis of 10 μ M II-R_p/S_p by 0.5 nM PTE_I106A_F132A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of II-R_p after irradiation at 365 nm. **C** Time course for the hydrolysis of 10 μ M II-R_p/S_p by 15 nM PTE_I106A_F132A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of II-S_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of II-R_p (red) were not considered in the fit. **D** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H257ONBY *as isolated*. Enzymatic hydrolysis of 10 μ M substrate II was initiated by addition of 15 nM PTE_I106A_F132A_H257ONBY and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorbance upon addition of PTE_G60A indicates that II-R_p is preferentially hydrolyzed. **E** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μ M substrate II was initiated by addition of 0.5 nM PTE_I106A_F132A_H257ONBY and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorbance upon addition of PTE_G60A indicates that II-S_p is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

Substrate III

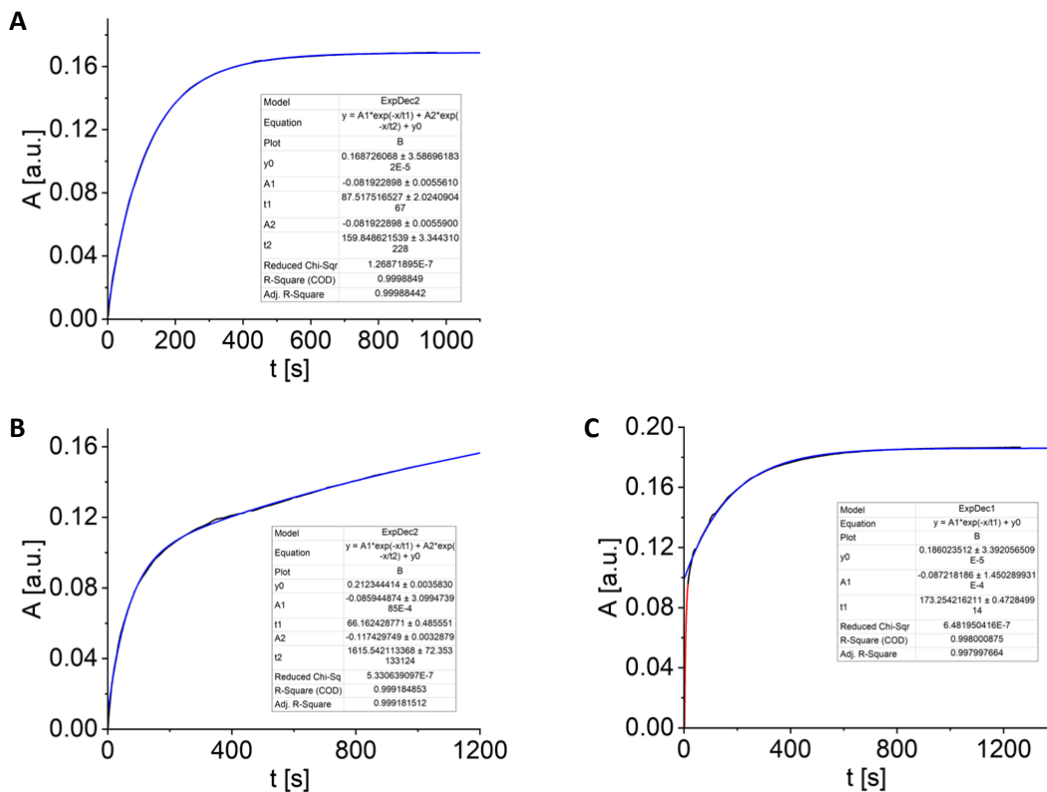


Figure S 54: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM III-S_P/R_P by 15 nM PTE_I106A_F132A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of III-S_P/R_P. **B** Time course for the hydrolysis of 10 μM III-R_P/S_P by 0.5 nM PTE_I106A_F132A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of III-R_P after irradiation at 365 nm. **C** Time course for the hydrolysis of 10 μM III-R_P/S_P by 5 nM PTE_I106A_F132A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of III-S_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of III-R_P (red) were not considered in the fit.

Substrate IV

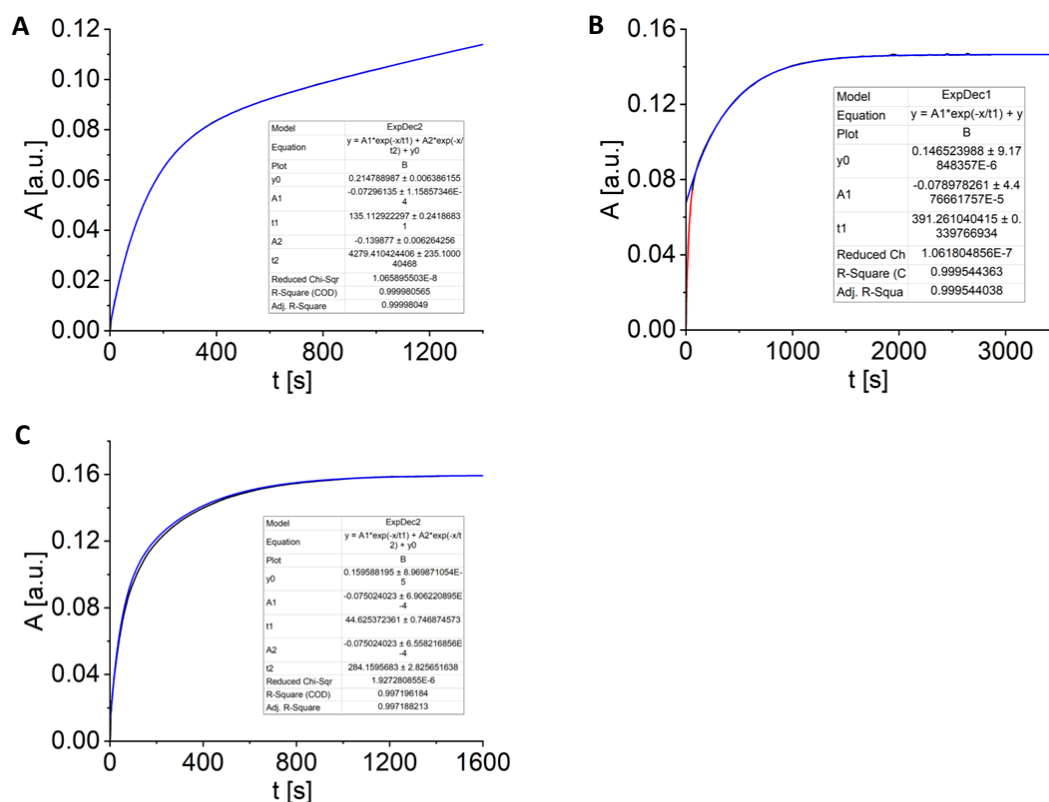


Figure S 55: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μ M IV-S_P/R_P by 400 nM PTE_I106A_F132A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of IV-S_P. **B** Time course for the hydrolysis of 10 μ M IV-S_P/R_P by 2 μ M PTE_I106A_F132A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of IV-R_P. Data points corresponding to the hydrolysis of IV-S_P (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μ M IV-S_P/R_P by 10 nM PTE_I106A_F132A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of IV-S_P/R_P after irradiation at 365 nm.

Substrate V

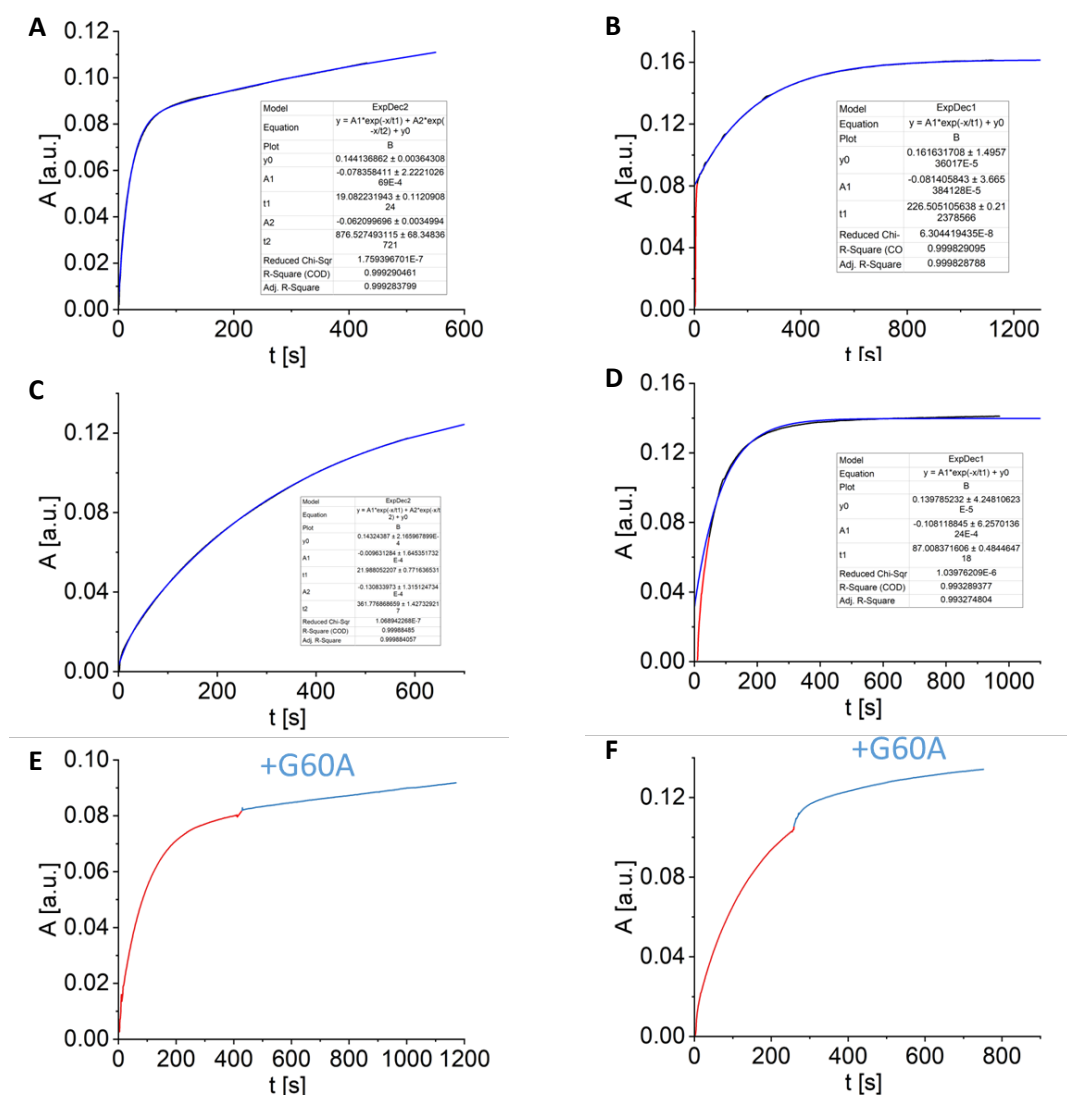


Figure S 56: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM V-S_P/R_P by 400 nM PTE_I106A_F132A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of V-S_P. **B** Time course for the hydrolysis of 10 μM V-S_P/R_P by 3 μM PTE_I106A_F132A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of V-R_P. Data points corresponding to the hydrolysis of V-S_P (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM V-S_P/R_P by 20 nM PTE_I106A_F132A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of V-S_P after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM V-S_P/R_P by 100 nM PTE_I106A_F132A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257ONBY for the hydrolysis of V-R_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of V-S_P (red) were not considered in the fit. **E** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H257ONBY *as isolated*. Enzymatic hydrolysis of 10 μM substrate V was initiated by addition of 100 nM PTE_I106A_F132A_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that IV-S_P is preferentially hydrolyzed. **F** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μM substrate V was initiated by addition of 30 nM PTE_I106A_F132A_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected upon addition of PTE_G60A, implying that V-S_P is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

1.1.13 PTE_I106A_F132A_H257Y

Substrate I

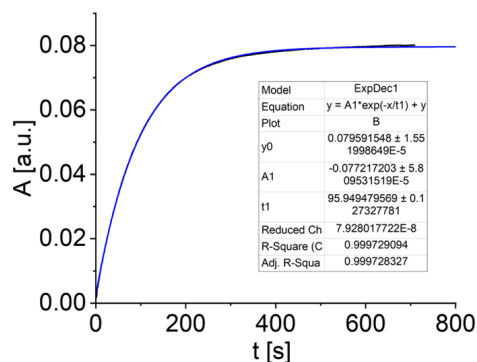


Figure S 57: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_F132A_H257Y

Time course for the hydrolysis of 5 μM substrate I by addition of 1 nM PTE_I106A_F132Y_H257Y (black). The data was fitted to a single exponential function.

Substrate II

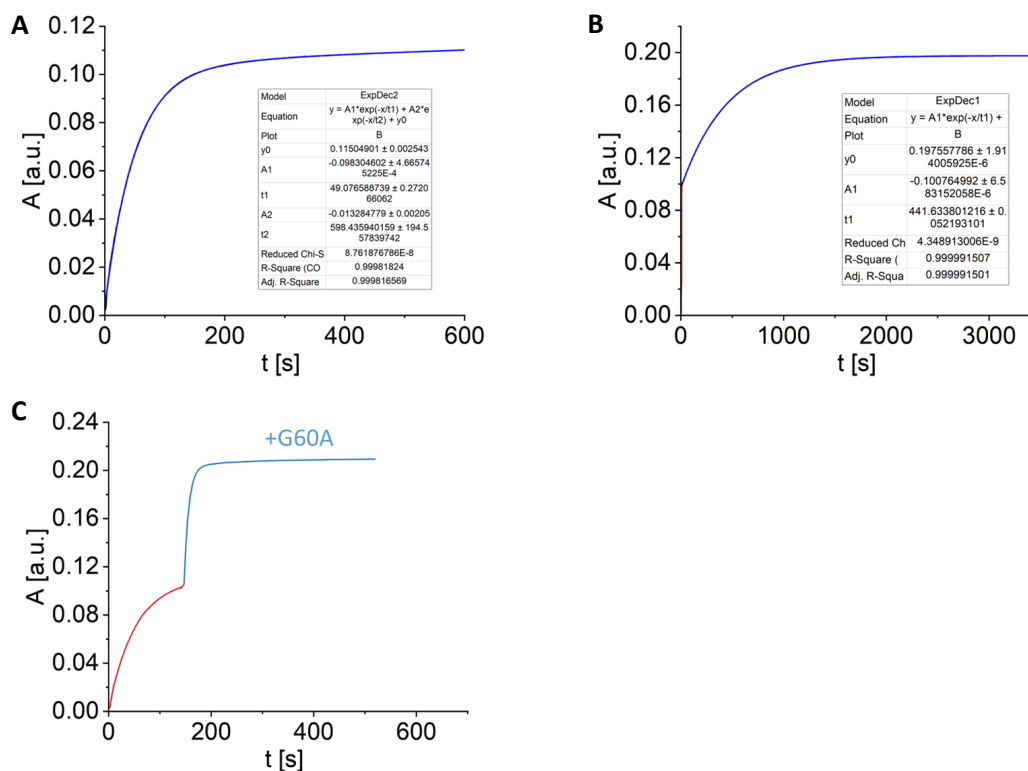


Figure S 58: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F123A_H257Y

A Time course for the hydrolysis of 10 μM II- R_p/S_p by 0.5 nM PTE_I106A_F132A_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y for the hydrolysis of II-R_p. **B** Time course for the hydrolysis of 10 μM II-R_p/S_p by 15 nM PTE_I106A_F132A_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y for the hydrolysis of II-S_p. Data points corresponding to the hydrolysis of II-R_p (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H257Y. Enzymatic hydrolysis of 10 μM substrate II was initiated by addition of 0.5 nM PTE_I106A_F132A_H257Y and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorbation upon addition of PTE_G60A indicates that II-R_p is preferentially hydrolyzed.

Substrate III

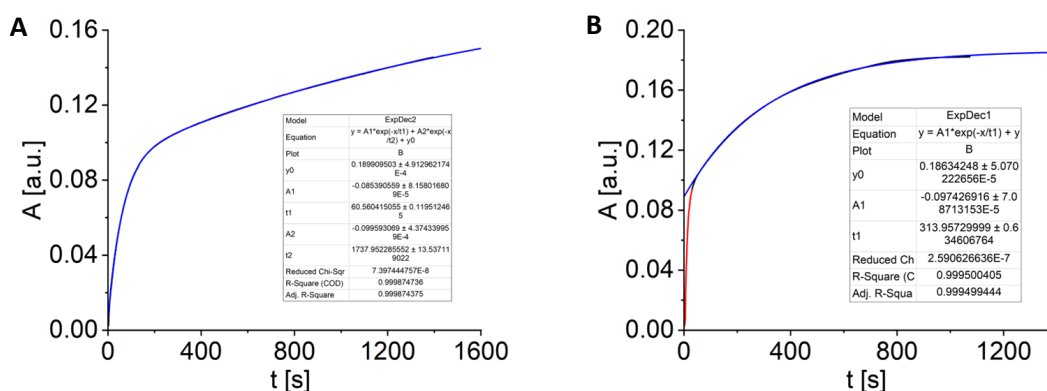


Figure S 59: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_H257Y

A Time course for the hydrolysis of 10 μ M III-R_p/S_p by 0.5 nM PTE_I106A_F132A_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y for the hydrolysis of III-R_p. **B** Time course for the hydrolysis of 10 μ M III-R_p/S_p by 5 nM PTE_I106A_F132A_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y for the hydrolysis of III-S_p. Data points corresponding to the hydrolysis of III-R_p (red) were not considered in the fit.

Substrate IV

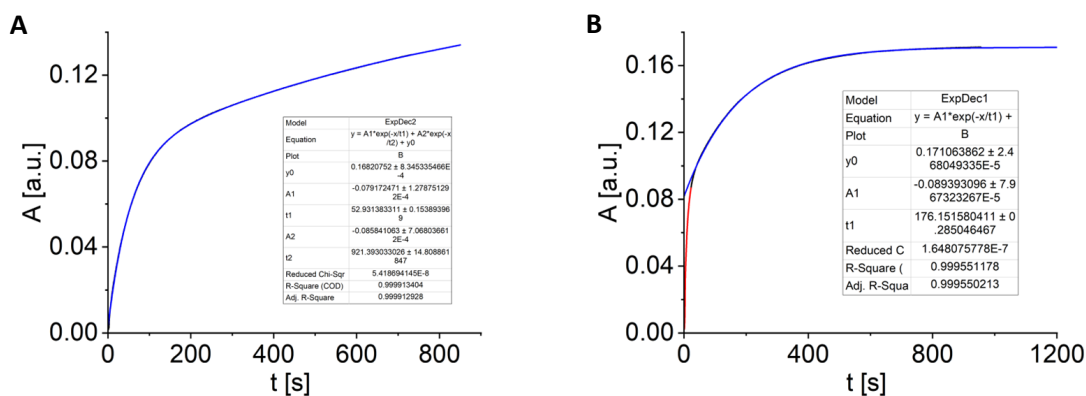


Figure S 60: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_H257Y

A Time course for the hydrolysis of 10 μ M IV-S_p/R_p by 100 nM PTE_I106A_F132A_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y for the hydrolysis of IV-S_p. **B** Time course for the hydrolysis of 10 μ M IV-S_p/R_p by 500 nM PTE_I106A_F132A_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y for the hydrolysis of IV-R_p. Data points corresponding to the hydrolysis of IV-S_p (red) were not considered in the fit.

Substrate V

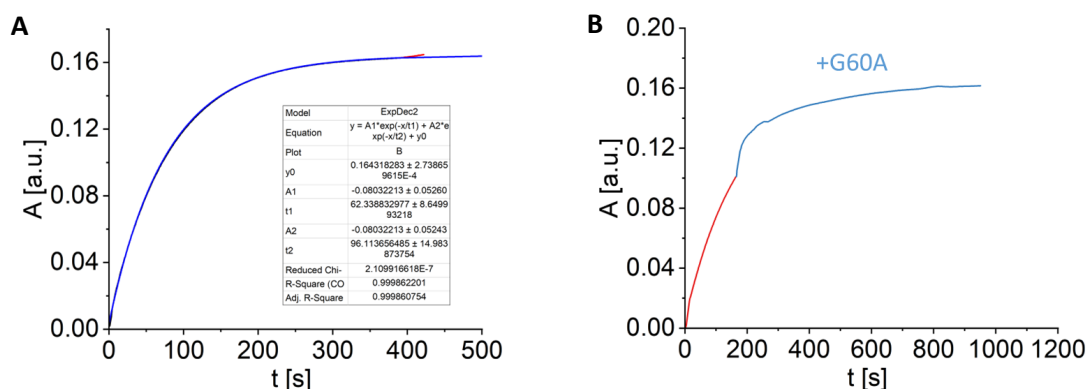


Figure S 61: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_H257Y

A Time course for the hydrolysis of 10 μ M V-S_P/R_P by 100 nM PTE_I106A_F132A_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y for the hydrolysis of V-S_P/R_P. **B** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H257Y. Enzymatic hydrolysis of 10 μ M substrate V was initiated by addition of 40 nM PTE_I106A_F132A_H257Y and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that V-S_P is preferentially hydrolyzed.

1.1.14 PTE_I106A_S308A_H257ONBY

Substrate I

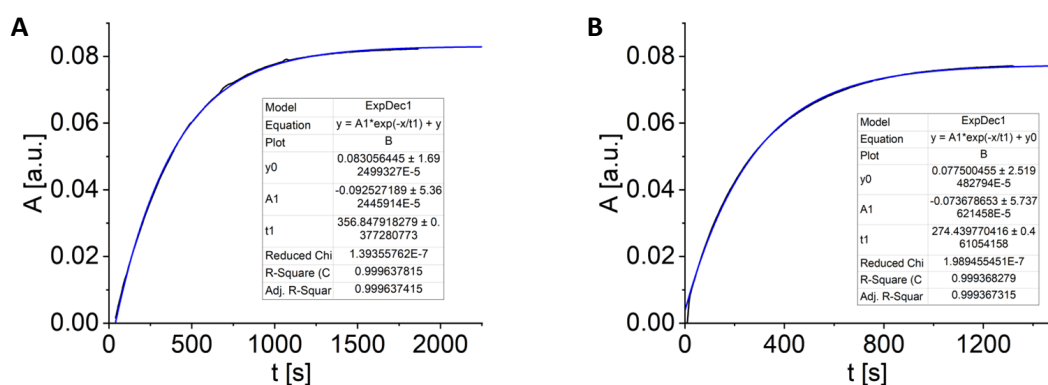


Figure S 62: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 5 μ M substrate I by 10 nM PTE_I106A_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential fit (blue). **B** Time course for the hydrolysis of 5 μ M substrate I by 2 nM PTE_I106A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential fit (blue).

Substrate II

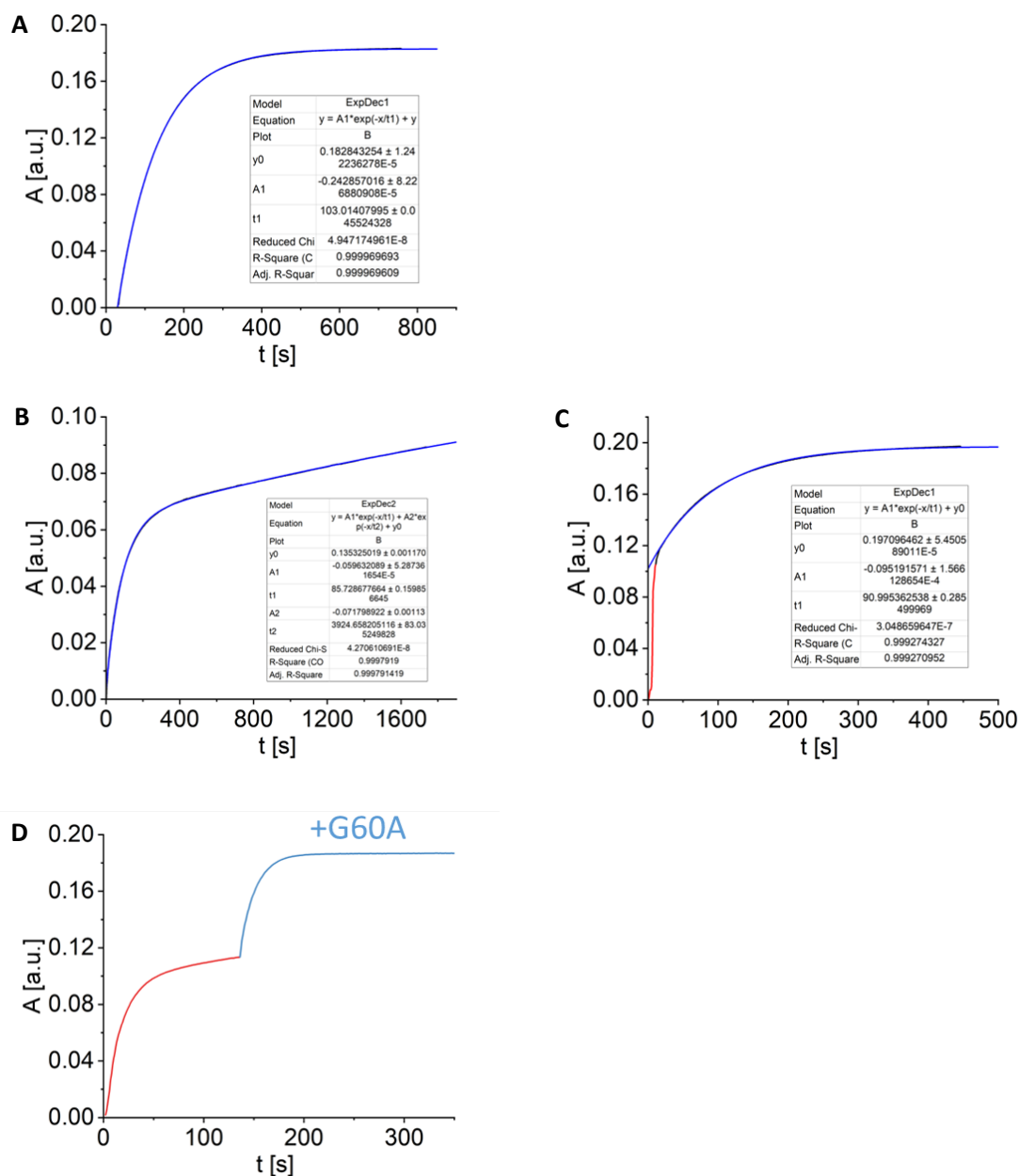


Figure S 63: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM II-R_P/S_P by 15 nM PTE_I106A_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of II-R_P/S_P. **B** Time course for the hydrolysis of 10 μM II-R_P/S_P by 0.5 nM PTE_I106A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of II-R_P after irradiation at 365 nm. **C** Time course for the hydrolysis of 10 μM II-R_P/S_P by 10 nM PTE_I106A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of II-S_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of II-R_P (red) were not considered in the fit. **D** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_S308A_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μM substrate II was initiated by addition of 1 nM PTE_I106A_S308A_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorption upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed.

Substrate III

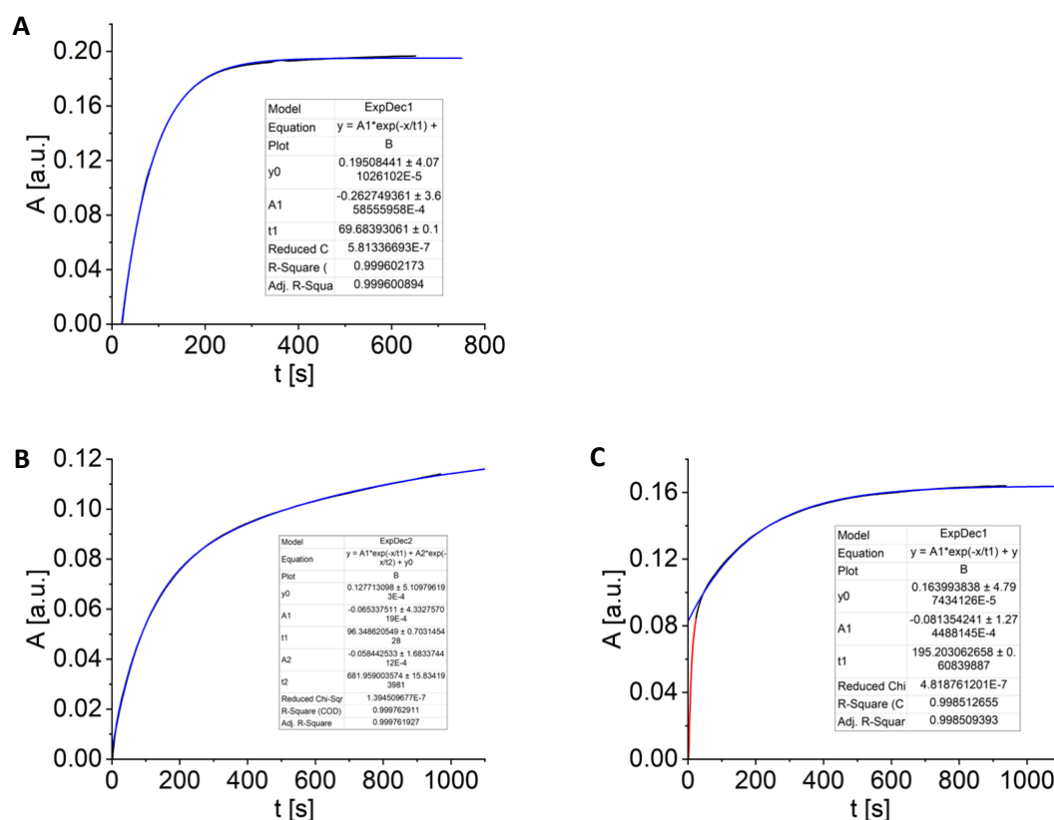


Figure S 64: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of $10 \mu\text{M}$ III-S_P/R_P by 15 nM PTE_I106A_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of III-S_P/R_P. **B** Time course for the hydrolysis of $10 \mu\text{M}$ III-R_P/S_P by 0.5 nM PTE_I106A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of III-R_P after irradiation at 365 nm . **C** Time course for the hydrolysis of $10 \mu\text{M}$ III-R_P/S_P by 2 nM PTE_I106A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of III-S_P after irradiation at 365 nm . Data points corresponding to the hydrolysis of III-R_P (red) were not considered in the fit.

Substrate IV

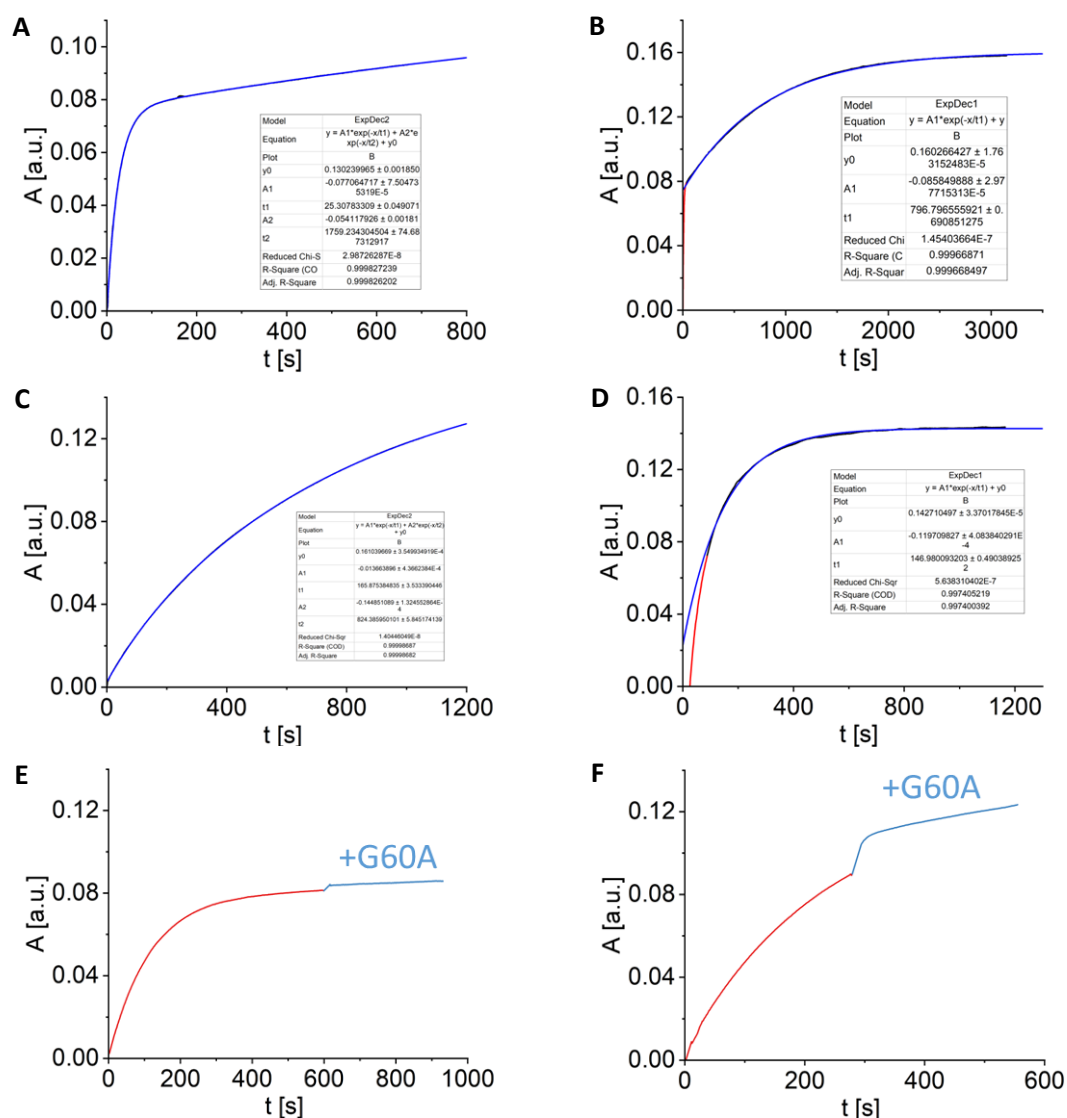


Figure S 65: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_S308_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM IV- S_p/R_p by 400 nM PTE_I106A_S308A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308_H257ONBY for the hydrolysis of IV- S_p . **B** Time course for the hydrolysis of 10 μM IV- S_p/R_p by 2 μM PTE_I106A_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of IV- R_p . Data points corresponding to the hydrolysis of IV- S_p (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM IV- S_p/R_p by 20 nM PTE_I106A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of IV- S_p after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM IV- S_p/R_p by 100 nM PTE_I106A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of IV- R_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of IV- S_p (red) were not considered in the fit. **E** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_S308A_H257ONBY *as isolated*. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 100 nM PTE_I106A_S308A_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that IV- S_p is preferentially hydrolyzed. **F** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_S308A_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 100 nM PTE_I106A_S308A_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. No significant increase in absorbance is observed upon addition of PTE_G60A, implying that IV- S_p is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

Substrate V

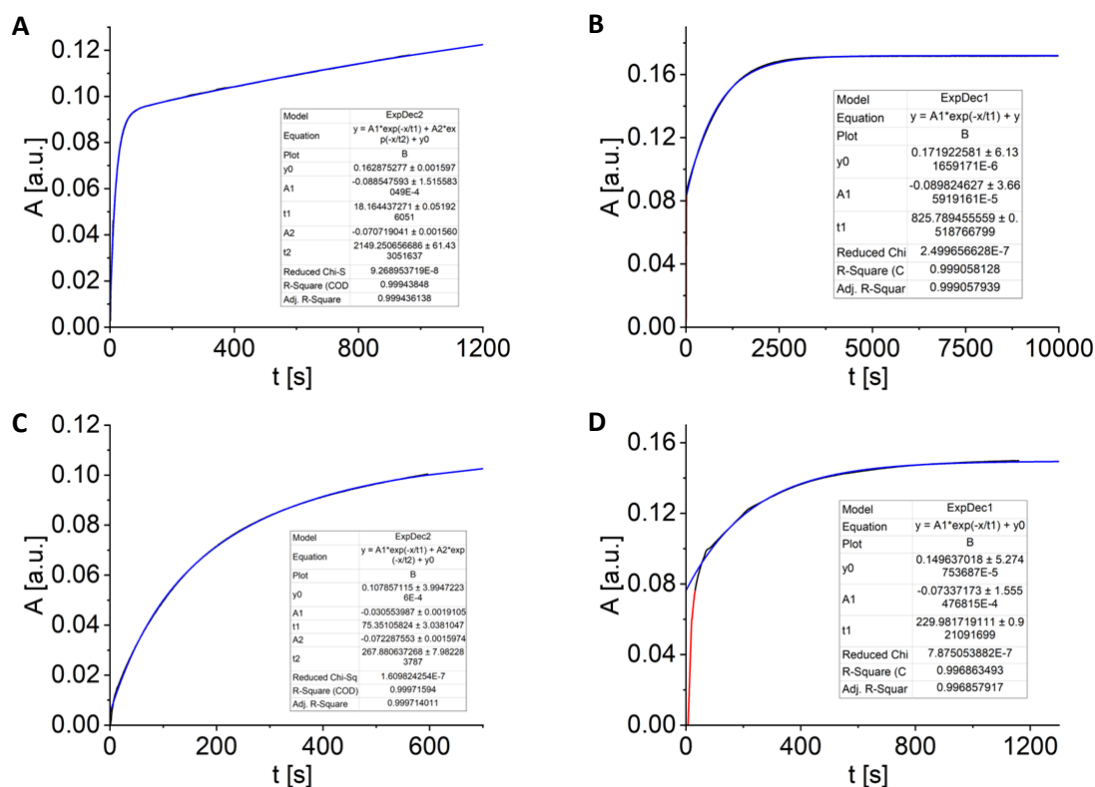


Figure S 66: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM V-S_p/R_p by 200 nM PTE_I106A_S308A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of V-S_p. **B** Time course for the hydrolysis of 10 μM V-S_p/R_p by 1 μM PTE_I106A_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of V-R_p. Data points corresponding to the hydrolysis of V-S_p (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM V-S_p/R_p by 20 nM PTE_I106A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of V-S_p after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM V-S_p/R_p by 100 nM PTE_I106A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_S308A_H257ONBY for the hydrolysis of V-R_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of V-S_p (red) were not considered in the fit.

1.1.15 PTE_I106A_H257Y_S308A

Substrate I

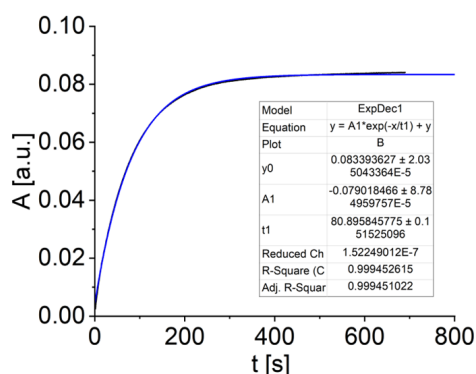


Figure S 67: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_H257Y_S308A

Time course for the hydrolysis of 5 μ M substrate I by addition of 2 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a single exponential function.

Substrate II

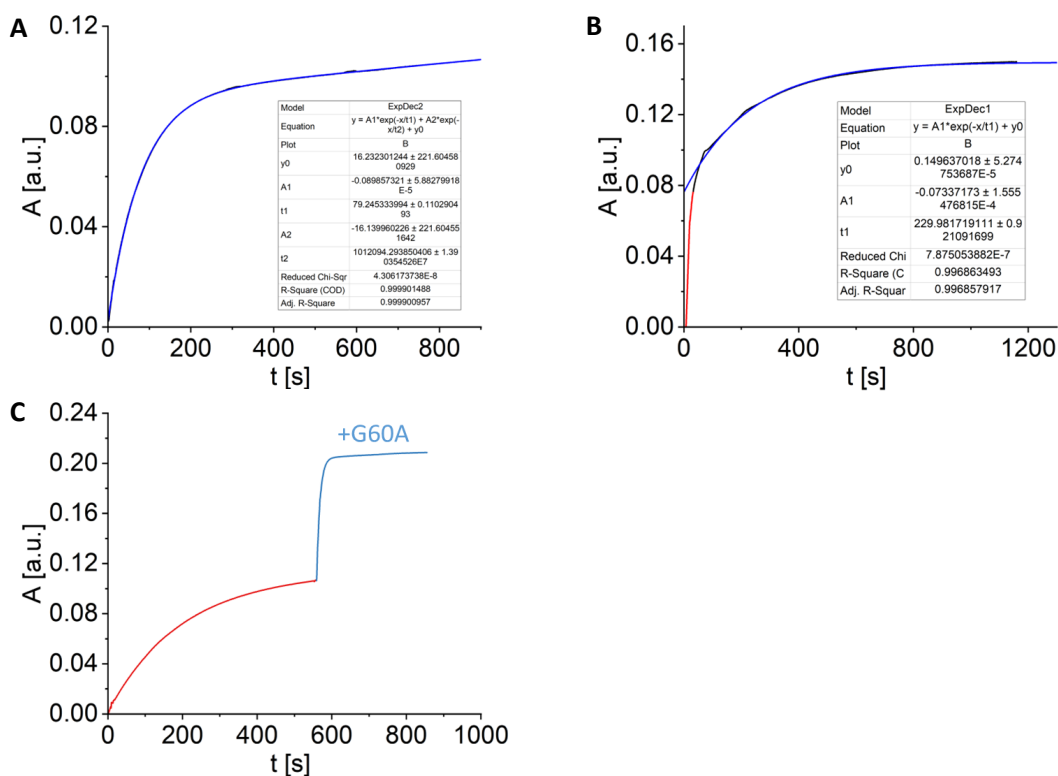


Figure S 68: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_H257Y_S308A

A Time course for the hydrolysis of 10 μ M II- R_p/S_p by 0.5 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for the hydrolysis of II-R_p. **B** Time course for the hydrolysis of 10 μ M II-R_p/S_p by 10 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y_S308A for the hydrolysis of II-S_p. Data points corresponding to the hydrolysis of II-R_p (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H257Y_S308A. Enzymatic hydrolysis of 10 μ M substrate II was initiated by addition of 0.5 nM PTE_I106A_H257Y_S308A and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorbance upon addition of PTE_G60A indicates that II-R_p is preferentially hydrolyzed.

Substrate III

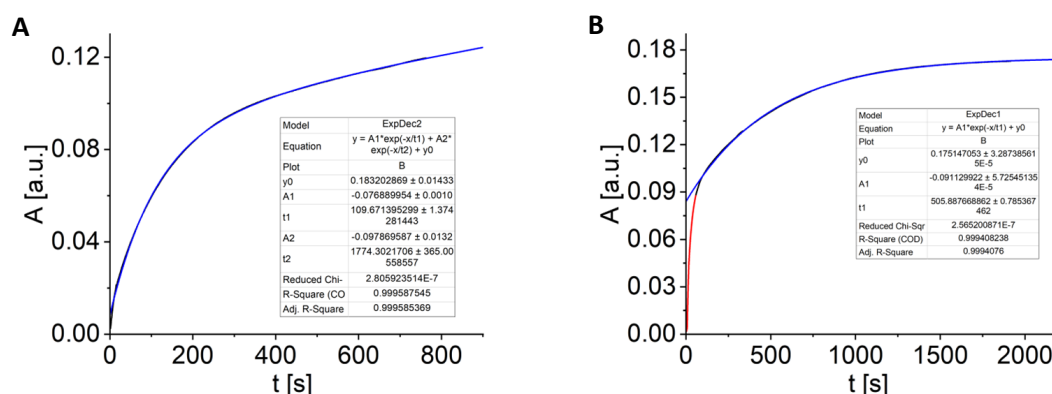


Figure S 69: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_H257Y_S308A

A Time course for the hydrolysis of 10 μM III-S_P/R_P by 0.5 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y_S308A for the hydrolysis of III-R_P. **B** Time course for the hydrolysis of 10 μM III-R_P/S_P by 2 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y_S308A for the hydrolysis of III-S_P. Data points corresponding to the hydrolysis of III-R_P (red) were not considered in the fit.

Substrate IV

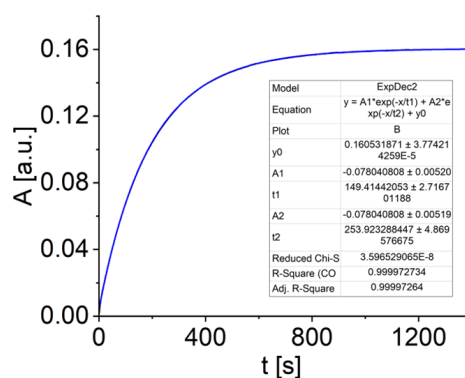


Figure S 70: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_H257Y_S308A

A Time course for the hydrolysis of 10 μM IV-S_P/R_P by 100 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y_S308A for the hydrolysis of IV-S_P/R_P.

Substrate V

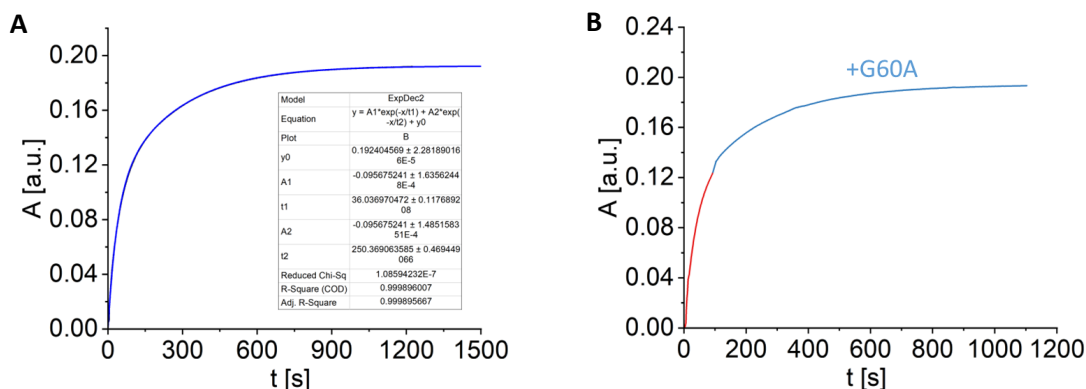


Figure S 71: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_H257Y_S308A

A Time course for the hydrolysis of 10 μM V-S_P/R_P by 200 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y_S308A for the hydrolysis of V-S_P/R_P. **B** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H257Y_S308A. Enzymatic hydrolysis of 10 μM substrate V was initiated by addition of 100 nM PTE_I106A_H257Y_S308A and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that V-S_P is preferentially hydrolyzed.

1.1.16 PTE_I106A_F132A_H254G_H257ONBY

Substrate I

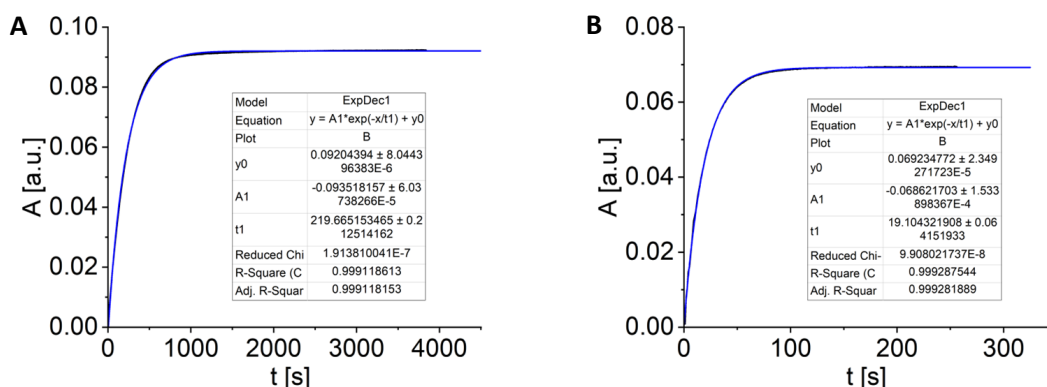


Figure S 72: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_F132A_H254G_H257ONBY before and after illumination

A Time course for the hydrolysis of 5 μM substrate I by 50 nM PTE_I106A_F132A_H254G_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue). **B** Time course for the hydrolysis of 5 μM substrate I by 20 nM PTE_I106A_F132A_H254G_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue).

Substrate II

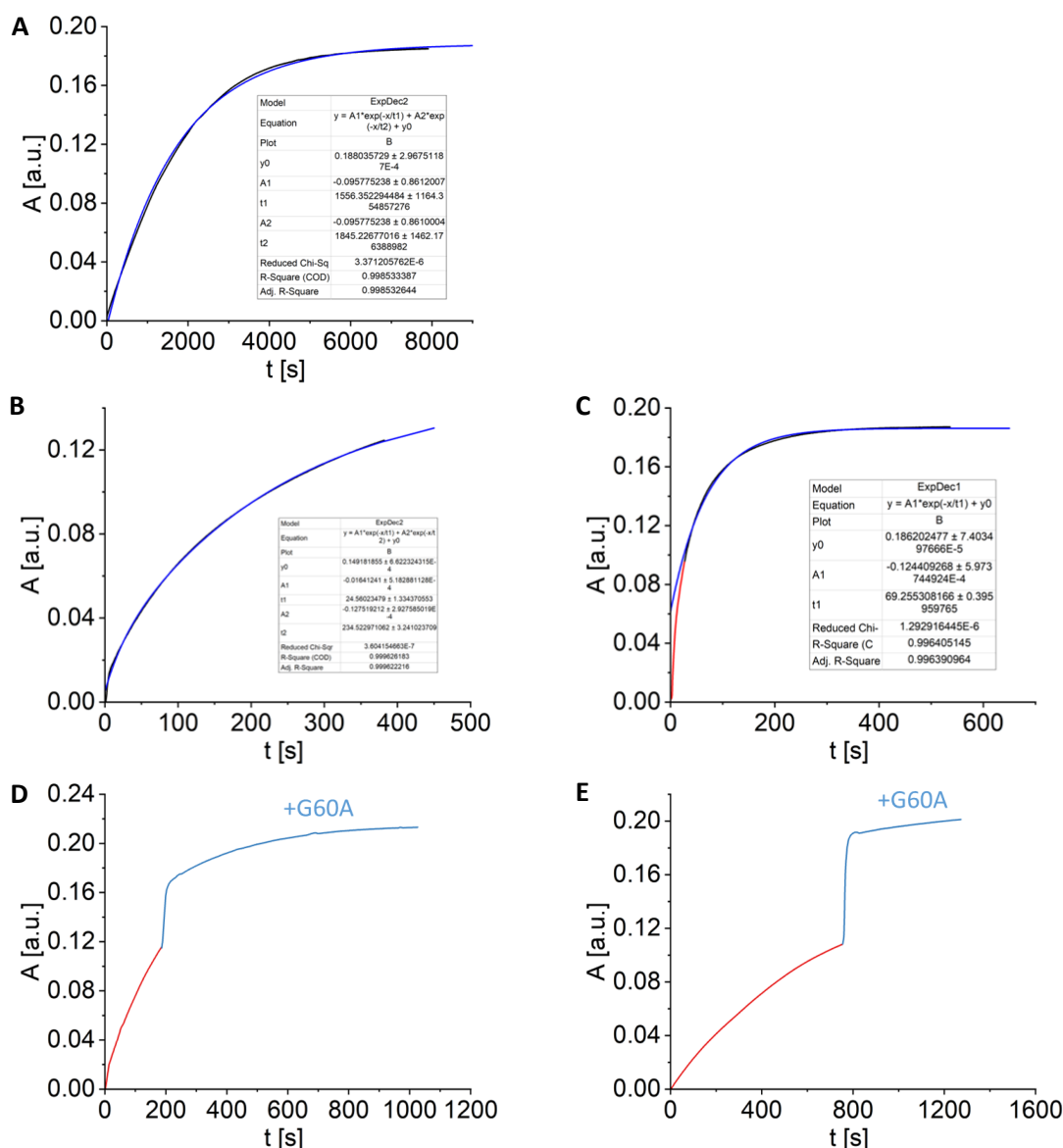


Figure S 73: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F132A_H254G_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μ M II-S_p/R_p by 2 nM PTE_I106A_F132A_H254G_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of II-R_p/S_p. **B** Time course for the hydrolysis of 10 μ M II-R_p/S_p by 1 nM PTE_I106A_F132A_H254G_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of II-R_p after irradiation at 365 nm. **C** Time course for the hydrolysis of 10 μ M II-R_p/S_p by 10 nM PTE_I106A_F132A_H254G_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of II-S_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of II-R_p (red) were not considered in the fit. **D** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H254G_H257ONBY *as isolated*. Enzymatic hydrolysis of 10 μ M substrate II was initiated by addition of 4 nM PTE_I106A_F132A_H254G_H257ONBY and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorption upon addition of PTE_G60A indicates that II-R_p is preferentially hydrolyzed. **E** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H254G_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μ M substrate II was initiated by addition of 1 nM PTE_I106A_F132A_H254G_H257ONBY and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorption upon addition of PTE_G60A indicates that II-S_p is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

Substrate III

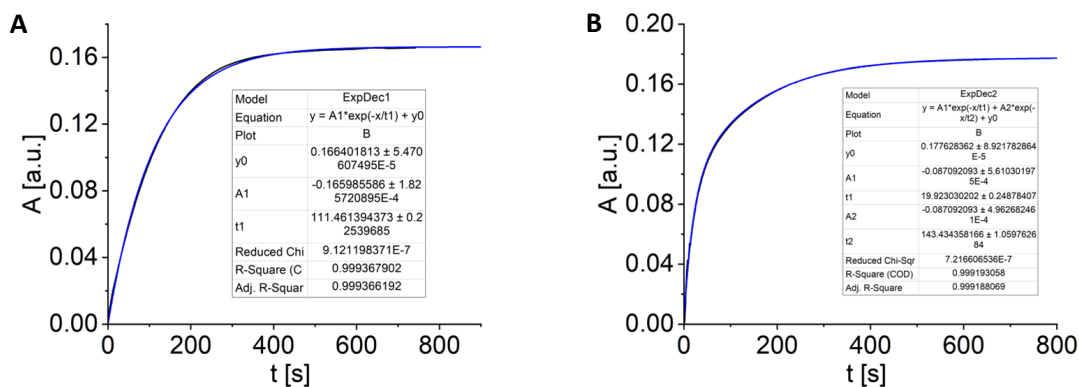


Figure S 74: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_H254G_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM III-R_p/S_p by 25 nM PTE_I106A_F132A_H254G_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of III-S_p/R_p. **B** Time course for the hydrolysis of 10 μM III-R_p/S_p by 10 nM PTE_I106A_F132A_H254G_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of III-R_p/S_p after irradiation at 365 nm.

Substrate IV

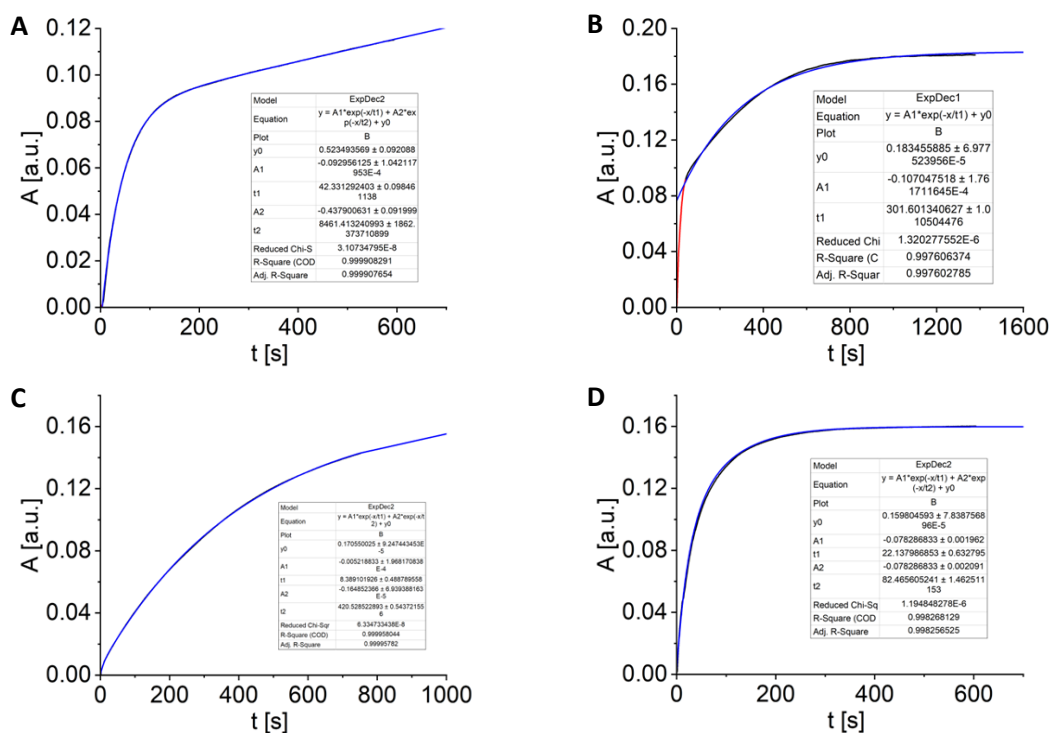


Figure S 75: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_H254G_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM IV-S_p/R_p by 300 nM PTE_I106A_F132A_H254G_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of IV-S_p. **B** Time course for the hydrolysis of 10 μM IV-S_p/R_p by 1.5 μM PTE_F132A_H254G_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of IV-R_p. Data points corresponding to the hydrolysis of IV-S_p (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM IV-S_p/R_p by 30 nM PTE_I106A_F132A_H254G_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of IV-S_p after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM IV-S_p/R_p by 200 nM PTE_I106A_F132A_H254G_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of IV-R_p after irradiation at 365 nm.

Substrate V

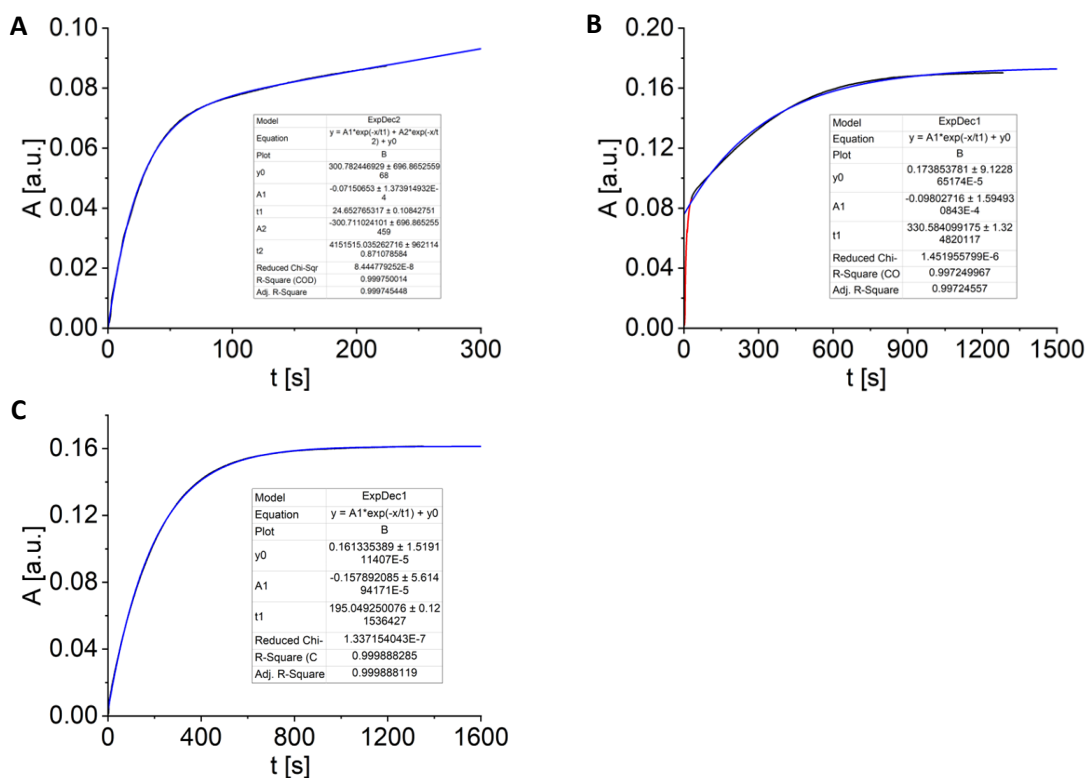


Figure S 76: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_H254G_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM V-S_P/R_P by 40 nM PTE_I106A_F132A_H254G_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of V-S_P. **B** Time course for the hydrolysis of 10 μM V-S_P/R_P by 1 μM PTE_I106A_F132A_H254G_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of V-R_P. Data points corresponding to the hydrolysis of V-S_P (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM V-S_P/R_P by 50 nM PTE_I106A_F132A_H254G_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257ONBY for the hydrolysis of V-S_P/R_P after irradiation at 365 nm.

1.1.17 PTE_I106A_F132A_H254G_H257Y

Substrate I

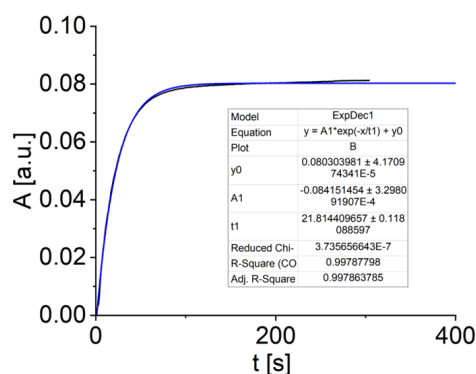


Figure S 77: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_F132A_H254G_H257Y

Time course for the hydrolysis of 5 μ M substrate I by addition of 5 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a single exponential function.

Substrate II

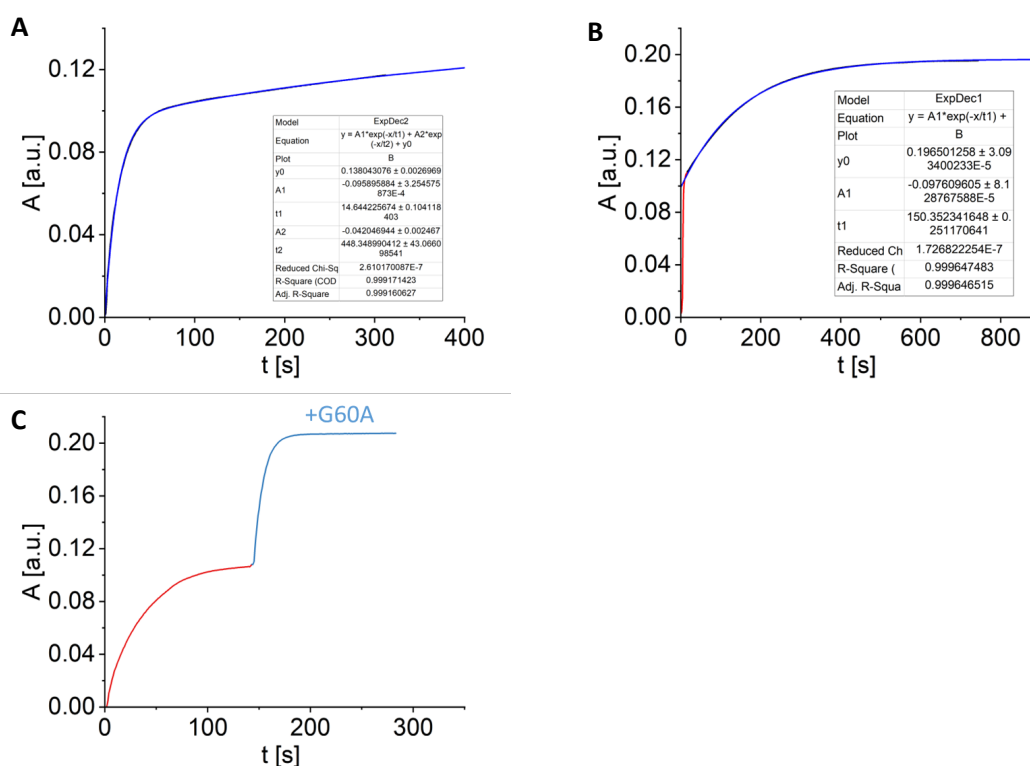


Figure S 78: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F132A_H254G_H257Y

A Time course for the hydrolysis of 10 μ M II- R_P/S_P by 10 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of II-R_P. **B** Time course for the hydrolysis of 10 μ M II-R_P/S_P by 100 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of II-S_P. Data points corresponding to the hydrolysis of II-R_P were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H254G_H257Y. Enzymatic hydrolysis of 10 μ M substrate II was initiated by addition of 10 nM PTE_I106A_F132A_H254G_H257Y and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorbance upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed.

Substrate III

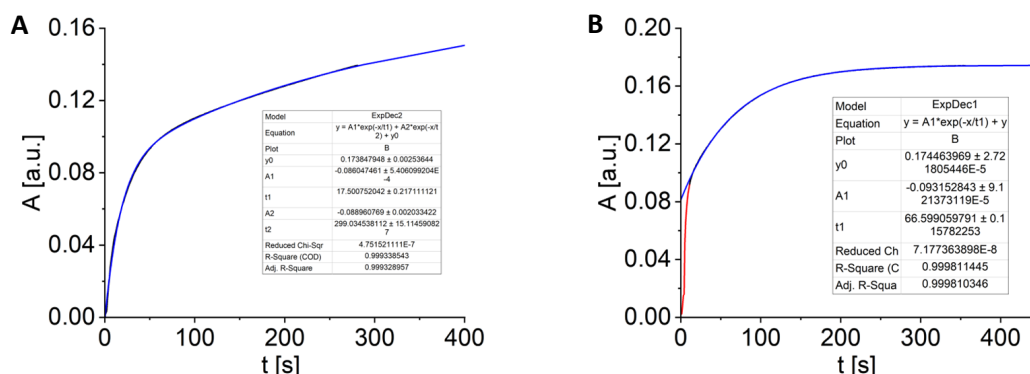


Figure S 79: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_H254G_H257Y

A Time course for the hydrolysis of 10 μ M III-S_P/R_P by 5 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of III-R_P. **B** Time course for the hydrolysis of 10 μ M III-R_P/S_P by 50 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of III-S_P. Data points corresponding to the hydrolysis of III-R_P (red) were not considered in the fit.

Substrate IV

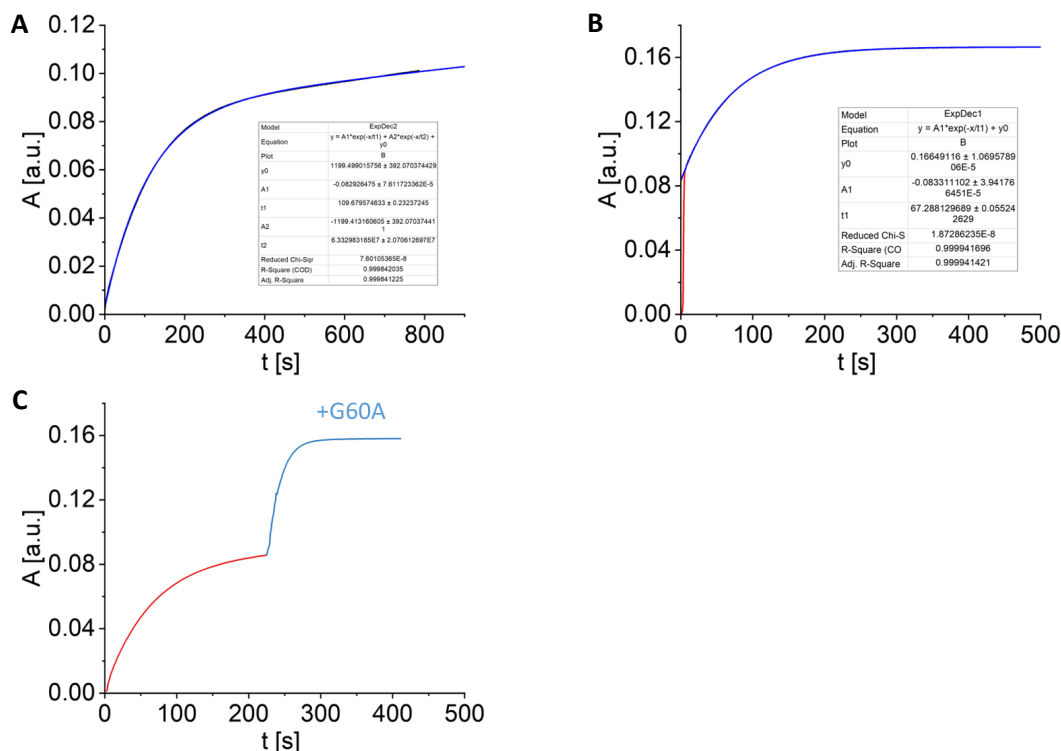


Figure S 80: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_H254G_H257Y

A Time course for the hydrolysis of 10 μ M IV-R_P/S_P by 50 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of IV-R_P. **B** Time course for the hydrolysis of 10 μ M IV-R_P/S_P by 2 μ M PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of IV-S_P. Data points corresponding to the hydrolysis of IV-R_P (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H254G_H257Y. Enzymatic hydrolysis of 10 μ M substrate IV was initiated by addition of 10 nM PTE_I106A_F132A_H254G_H257Y and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in upon addition of PTE_G60A indicates that IV-R_P is preferentially hydrolyzed.

Substrate V

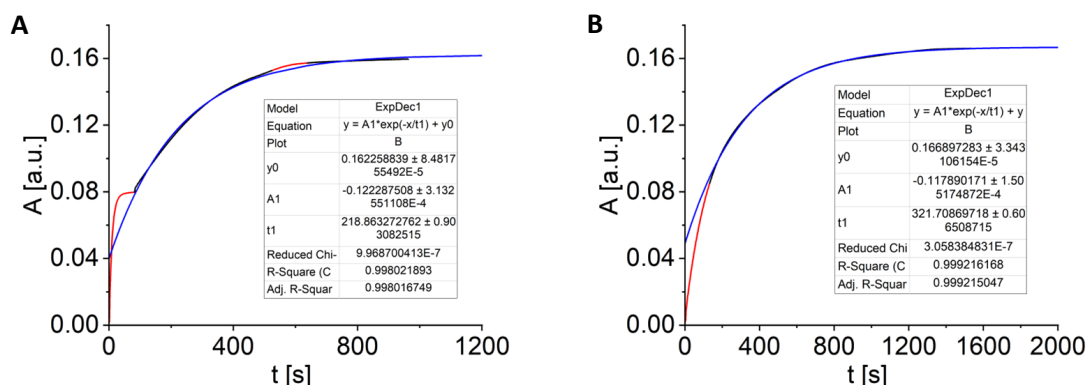


Figure S 81: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_H254G_H257Y

A Complementation assay with PTE_G60A to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of V-R_p. Enzymatic hydrolysis was initiated by addition of 15 nM PTE_G60A and 30 nM PTE_I106A_F132A_H254G_H257Y was added after one isomer was quantitatively hydrolyzed. The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of V-R_p. **B** Time course for the hydrolysis of 10 μM V-R_p/S_p by 80 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of V-S_p. Data points corresponding to the hydrolysis of V-R_p (red) were not considered in the fit.

1.1.18 PTE_I106A_H254G_S308A_H257ONBY

Substrate I

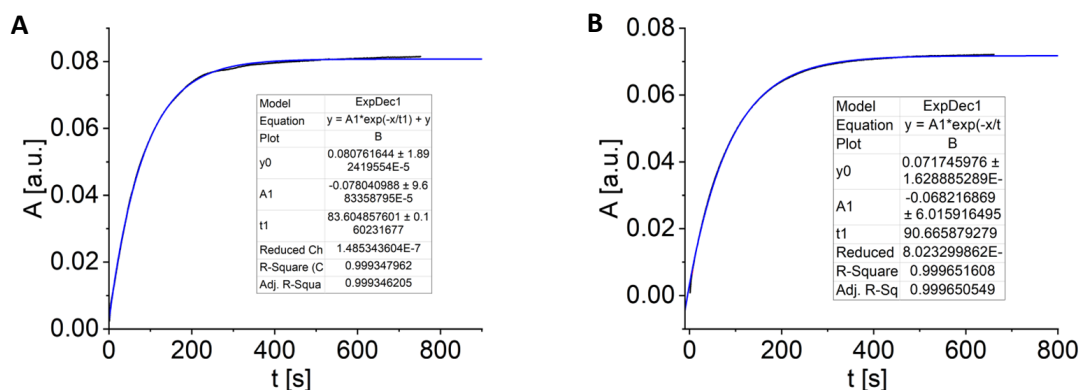


Figure S 82: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_H254G_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 5 μM substrate I by 5 nM PTE_I106A_H254G_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue). **B** Time course for the hydrolysis of 5 μM substrate I by 5 nM PTE_I106A_H254G_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue).

Substrate II

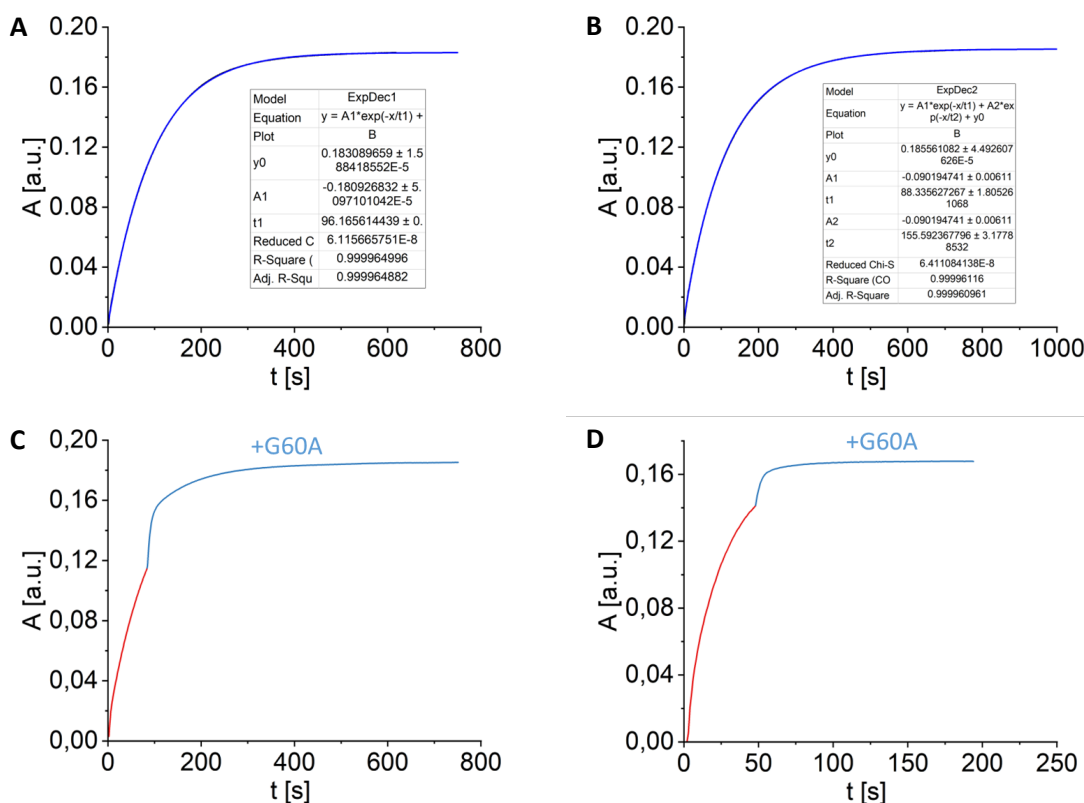


Figure S 83: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_H254G_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of $10 \mu\text{M}$ II-R_P/S_P by 2 nM PTE_I106A_H254G_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of II-S_P/R_P. **B** Time course for the hydrolysis of $10 \mu\text{M}$ II-R_P/S_P by 1.5 nM PTE_I106A_H254G_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of II-R_P/S_P after irradiation at 365 nm. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H254G_S308A_H257ONBY *as isolated*. Enzymatic hydrolysis of $10 \mu\text{M}$ substrate II was initiated by addition of 2 nM PTE_I106A_H254G_S308A_H257ONBY and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorption upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed. **D** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H254G_S308A_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of $10 \mu\text{M}$ substrate II was initiated by addition of 1 nM PTE_I106A_H254G_S308A_H257ONBY and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorption upon addition of PTE_G60A indicates that II-S_P is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

Substrate III

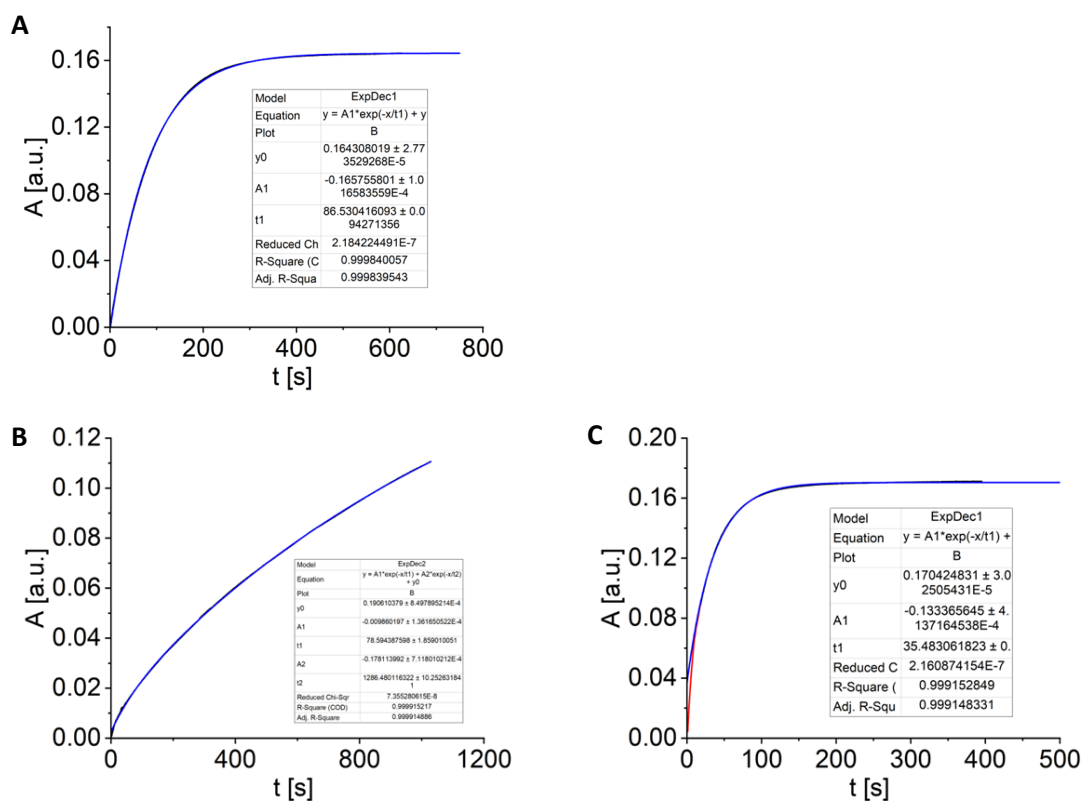


Figure S 84: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_H254G_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM III-R_p/S_p by 2 nM PTE_I106A_H254G_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of III-S_p/R_p. **B** Time course for the hydrolysis of 10 μM III-R_p/S_p by 0.5 nM PTE_I106A_H254G_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of III-R_p/S_p after irradiation at 365 nm. **C** Time course for the hydrolysis of 10 μM III-R_p/S_p by 1.5 nM PTE_I106A_H254G_S308A_H257ONBY after irradiation at 365 nm. The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of III-S_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of III-R_p (red) were not considered in the fit.

Substrate IV

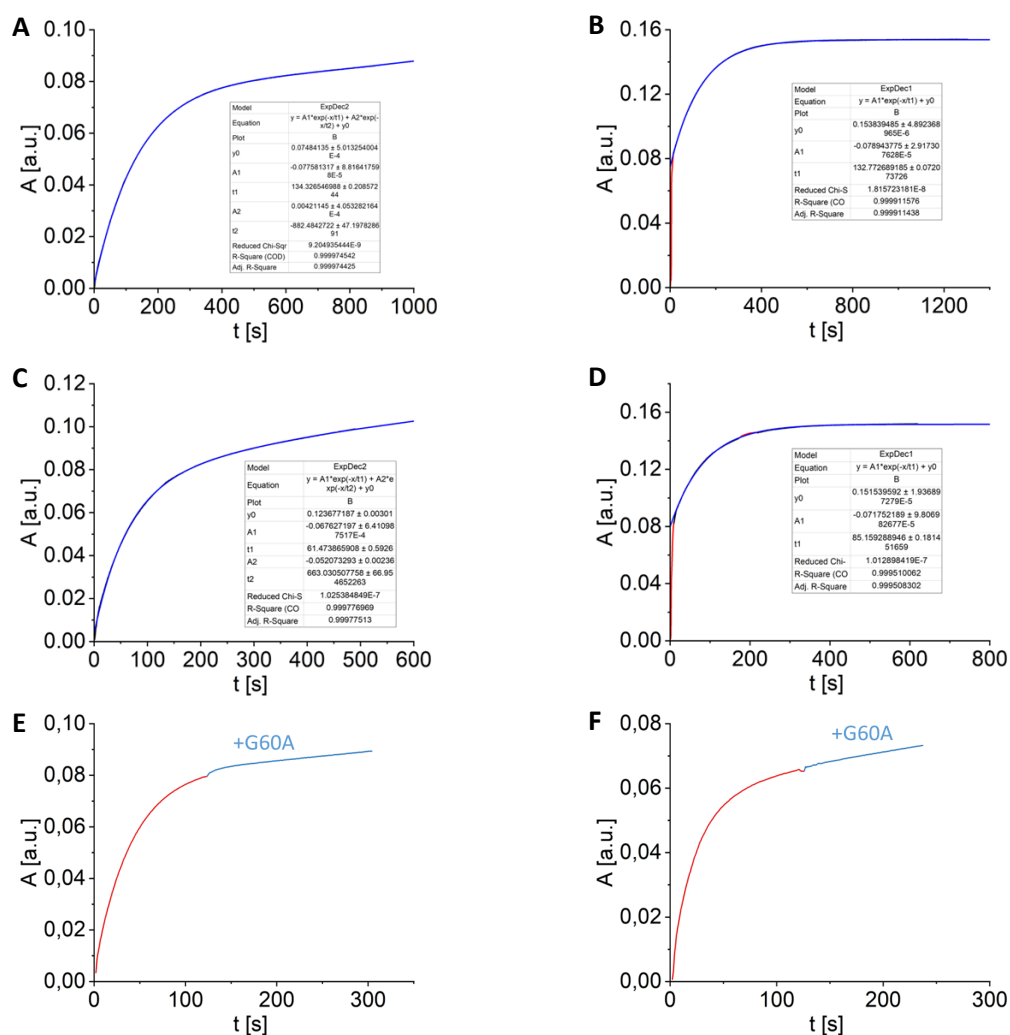


Figure S 85: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_H254G_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM IV- S_P /R $_P$ by 10 nM PTE_I106A_H254G_S308A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of IV- S_P . **B** Time course for the hydrolysis of 10 μM IV- S_P /R $_P$ by 500 nM PTE_I106A_H254G_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of IV-R $_P$. Data points corresponding to the hydrolysis of IV- S_P (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM IV- S_P /R $_P$ by 20 nM PTE_I106A_H254G_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of IV- S_P after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM IV- S_P /R $_P$ by 300 nM PTE_I106A_H254G_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of IV-R $_P$ after irradiation at 365 nm. Data points corresponding to the hydrolysis of IV- S_P (red) were not considered in the fit. **E** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H254G_S308A_H257ONBY *as isolated*. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 30 nM PTE_I106A_H254G_S308A_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that IV- S_P is preferentially hydrolyzed. **F** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H254G_S308A_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 30 nM PTE_I106A_H254G_S308A_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected upon addition of PTE_G60A, implying that IV- S_P is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

Substrate V

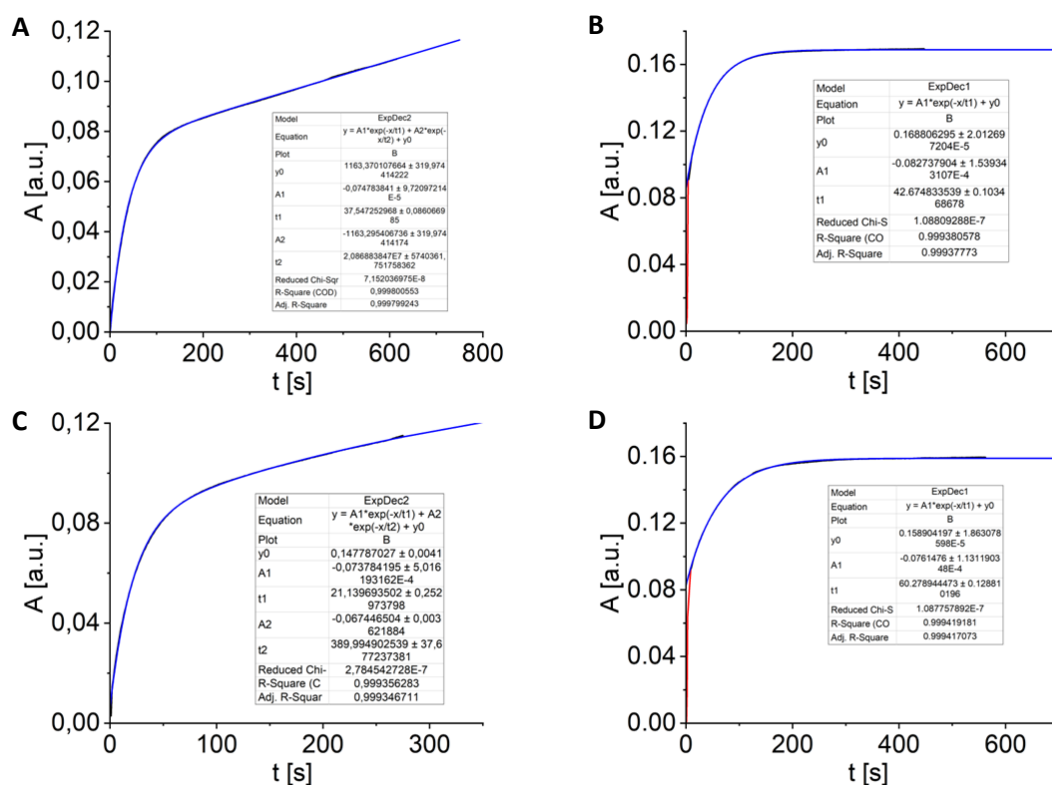


Figure S 86: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_H254G_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM V-S_P/R_P by 20 nM PTE_I106A_H254G_S308A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of V-S_P. **B** Time course for the hydrolysis of 10 μM V-S_P/R_P by 500 nM PTE_I106A_H254G_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of V-R_P. Data points corresponding to the hydrolysis of V-S_P (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM V-S_P/R_P by 10 nM PTE_I106A_H254G_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of V-S_P after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM V-S_P/R_P by 200 nM PTE_I106A_H254G_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_S308A_H257ONBY for the hydrolysis of V-R_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of V-S_P (red) were not considered in the fit.

1.1.19 PTE_I106A_H254G_H257Y_S308A

Substrate I

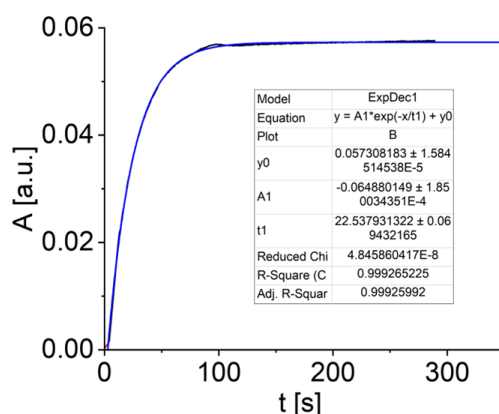


Figure S 87: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_H254G_H257Y_S308A

Time course for the hydrolysis of 5 μM substrate I by addition of 5 nM PTE_I106A_H257Y (black). The data was fitted to a single exponential function.

Substrate II

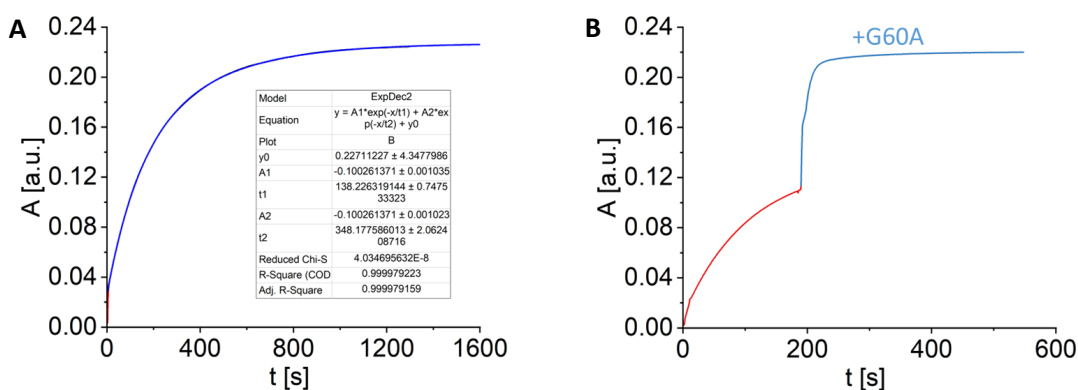


Figure S 88: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_H254G_H257Y_S308A

A Time course for the hydrolysis of 10 μM II- R_P/S_P by 1 nM PTE_I106A_H254G_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_H257Y_S308A for the hydrolysis of II-R_P/S_P. **B** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H254G_H257Y_S308A. Enzymatic hydrolysis of 10 μM substrate II was initiated by addition of 0.5 nM PTE_I106A_H254G_H257Y_S308A and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorbance upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed.

Substrate III

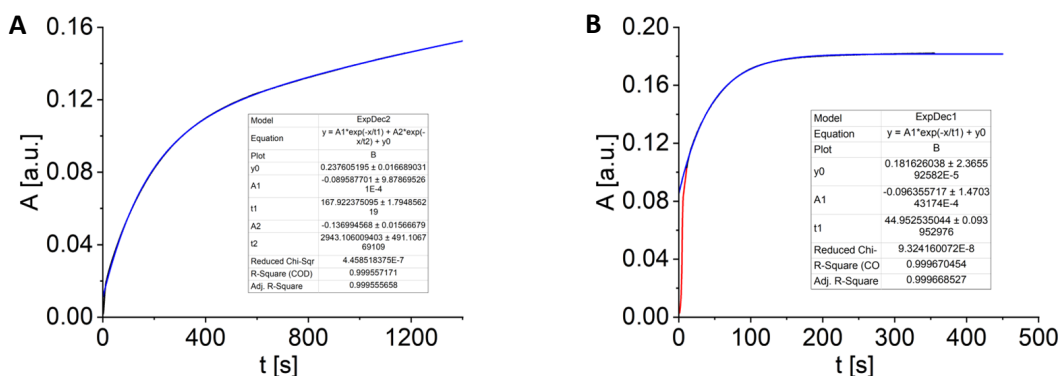


Figure S 89: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_H254G_H257Y_S308A

A Time course for the hydrolysis of 10 μM III- R_P /S P by 1.5 nM PTE_I106A_H254G_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_H257Y_S308A for the hydrolysis of III- R_P . **B** Time course for the hydrolysis of 10 μM III- R_P /S P by 15 nM PTE_I106A_H254G_H257Y_S308A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_H257Y_S308A for the hydrolysis of III- S_P . Data points corresponding to the hydrolysis of III- R_P (red) were not considered in the fit.

Substrate IV

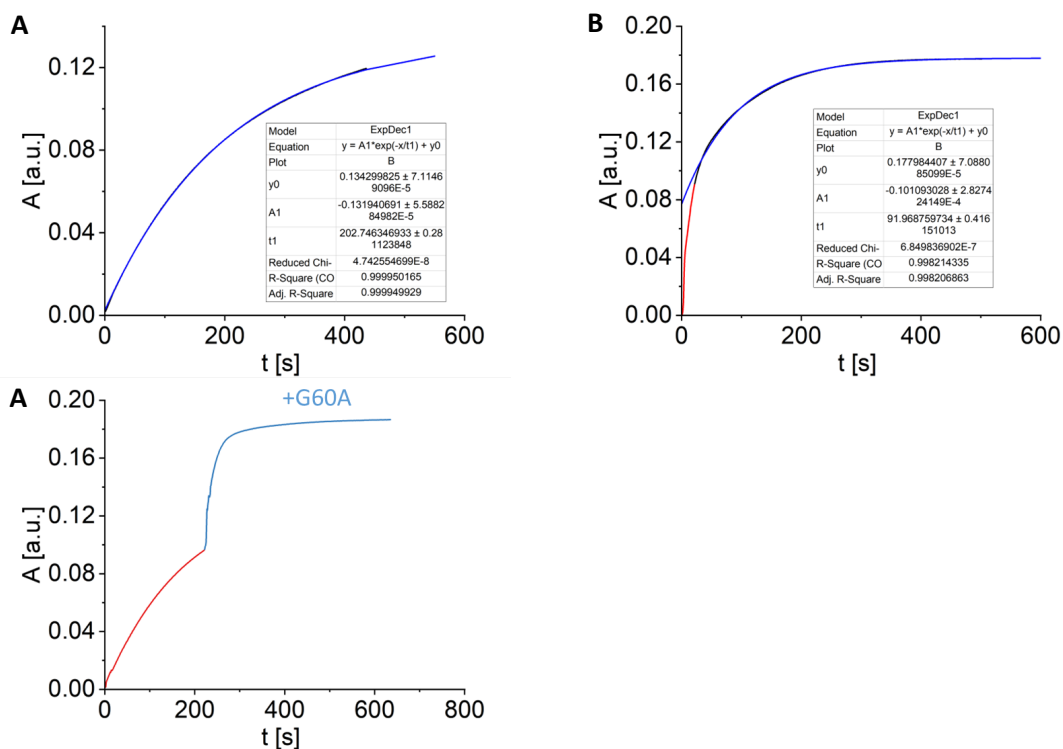


Figure S 90: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_H254G_H257Y_S308A

A Time course for the hydrolysis of 10 μM IV- R_P /S P by 15 nM PTE_I106A_H254G_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_H257Y_S308A for the hydrolysis of IV- R_P . **B** Time course for the hydrolysis of 10 μM IV- R_P /S P by 100 nM PTE_I106A_H254G_H257Y_S308A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_H257Y_S308A for the hydrolysis of IV- S_P . Data points corresponding to the hydrolysis of IV- R_P (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_H254G_H257Y_S308A. Enzymatic hydrolysis of 10 μM substrate IV was initiated by addition of 30 nM PTE_I106A_H254G_H257Y_S308A and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in upon addition of PTE_G60A indicates that IV- R_P is preferentially hydrolyzed.

Substrate V

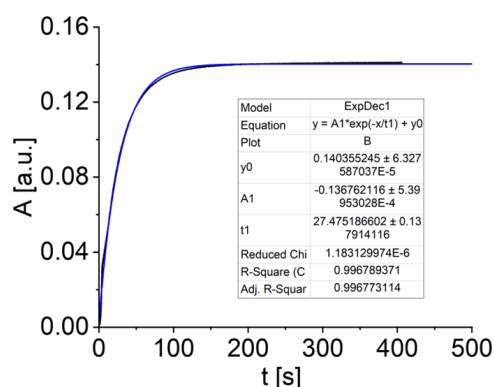


Figure S 91: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_H254G_H257Y_S308A

A Time course for the hydrolysis of 10 μM V- R_p/S_p by 100 nM PTE_I106A_H254G_H257Y_S308A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H254G_H257Y_S308A for the hydrolysis of V-R_p/S_p.

1.1.20 PTE_I106A_F132A_S308A_H257ONBY

Substrate I

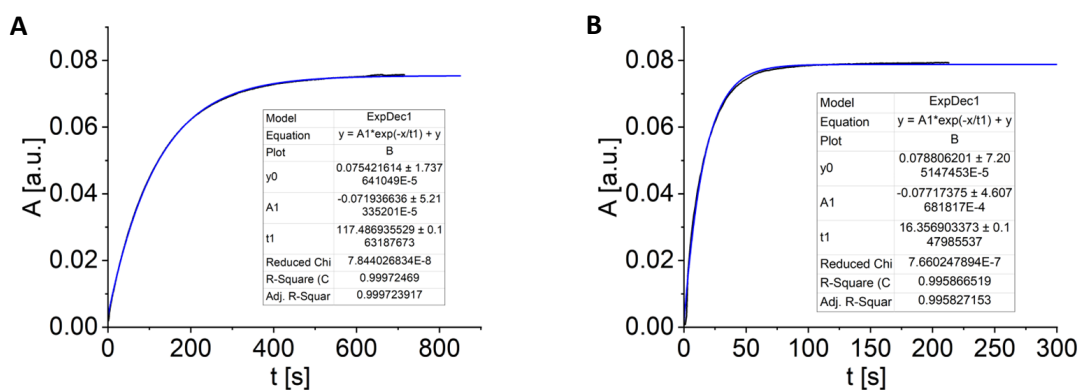


Figure S 92: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_F132A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 5 μM substrate I by 50 nM PTE_I106A_F132A_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue). **B** Time course for the hydrolysis of 5 μM substrate I by 10 nM PTE_I106A_F132A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue).

Substrate II

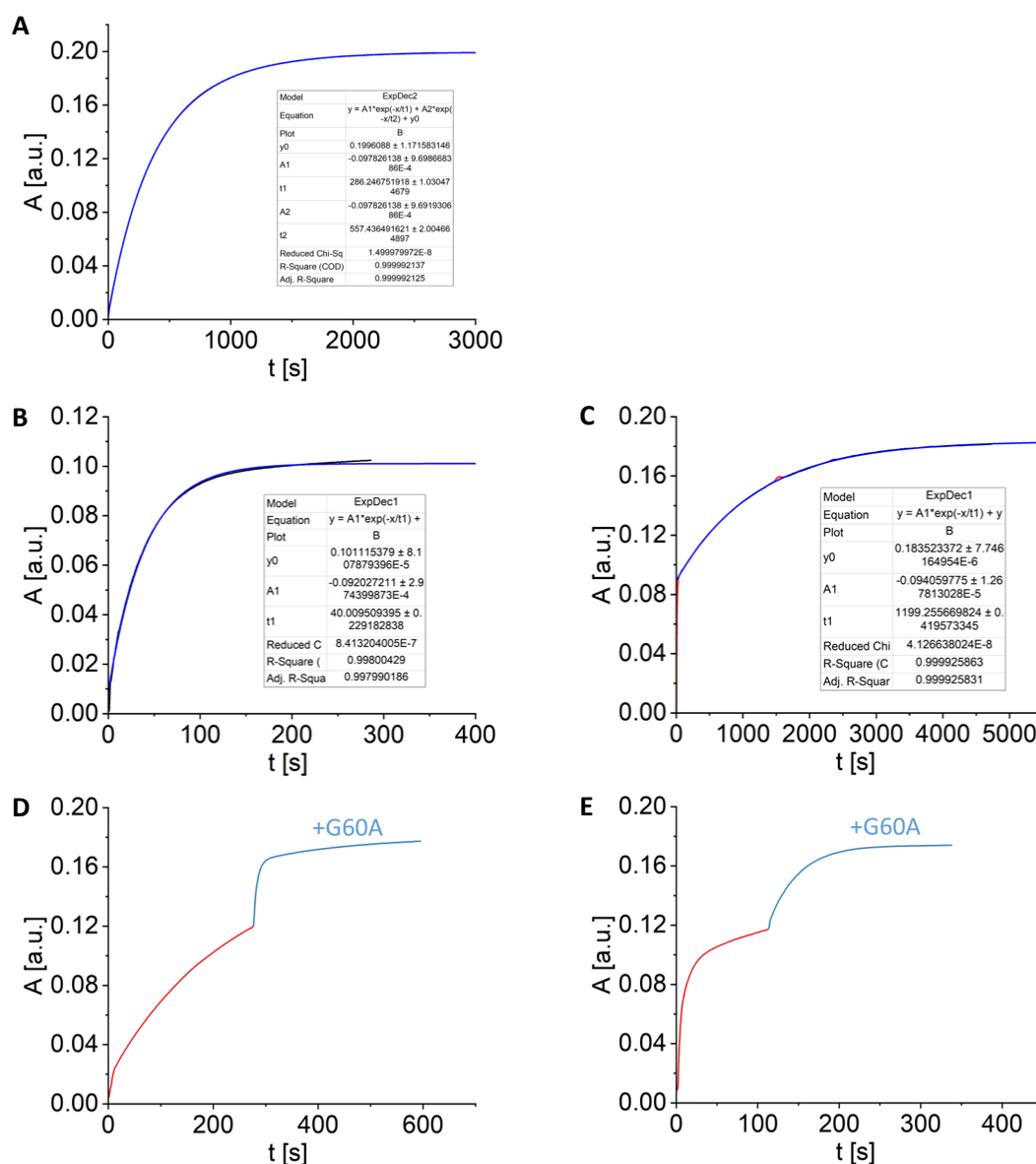


Figure S 93: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F132A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM II-S_P/R_P by 5 nM PTE_I106A_F132A_S308A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of II-S_P/R_P. **B** Time course for the hydrolysis of 10 μM II-R_P/S_P by 0.5 nM PTE_I106A_F132A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of II-R_P after irradiation at 365 nm. **C** Time course for the hydrolysis of 10 μM II-R_P/S_P by 2 nM PTE_I106A_F132A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of II-S_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of II-R_P (red) were not considered in the fit. **D** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_S308A_H257ONBY *as isolated*. Enzymatic hydrolysis of 10 μM substrate II was initiated by addition of 5 nM PTE_I106A_F132A_S308A_H257ONBY and PTE_G60A was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorbance upon addition of PTE_G60A (5 nM) indicates that II-R_P is preferentially hydrolyzed. **E** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_S308A_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μM substrate II was initiated by addition of 5 nM PTE_I106A_F132A_S308A_H257ONBY and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorbance upon addition of PTE_G60A indicates that II-S_P is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

Substrate III

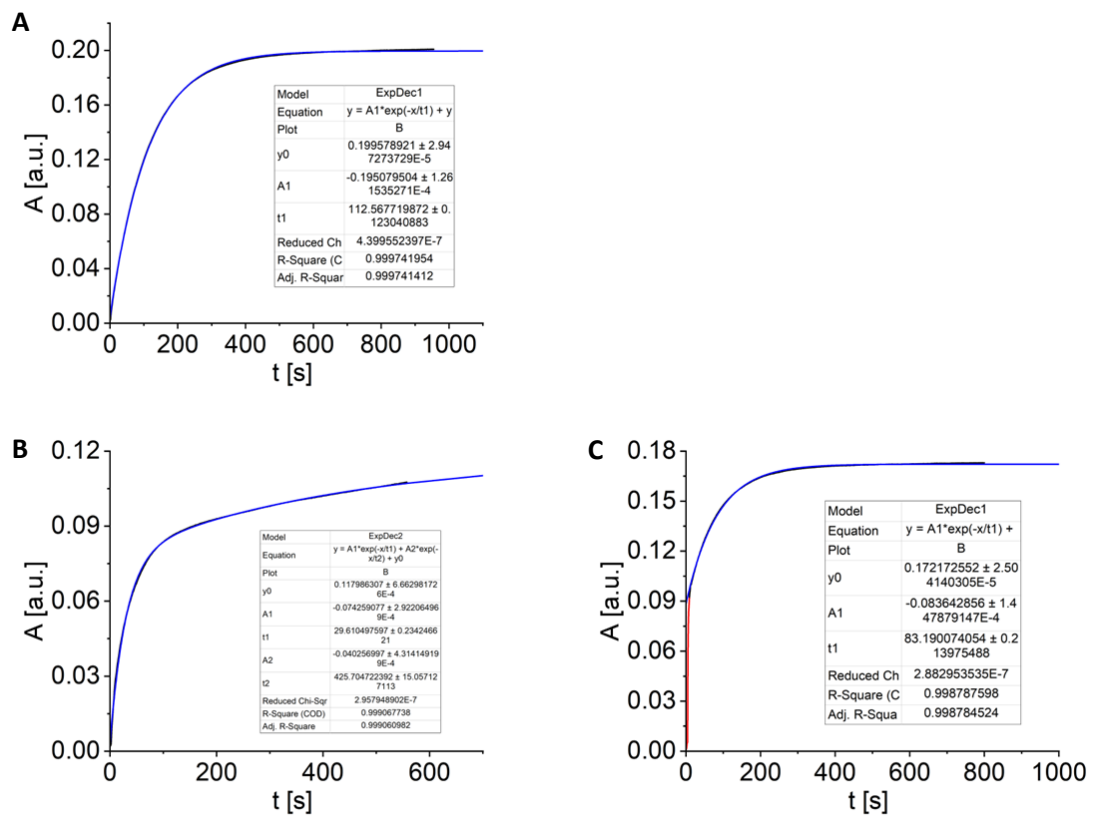


Figure S 94: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μ M III-R_P/S_P by 10 nM PTE_I106A_F132A_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of III-S_P/R_P. **B** Time course for the hydrolysis of 10 μ M III-R_P/S_P by 1 nM PTE_I106A_F132A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of III-R_P/S_P after irradiation at 365 nm. **C** Time course for the hydrolysis of 10 μ M III-R_P/S_P by 10 nM PTE_I106A_F132A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of III-S_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of III-R_P (red) were not considered in the fit.

Substrate IV

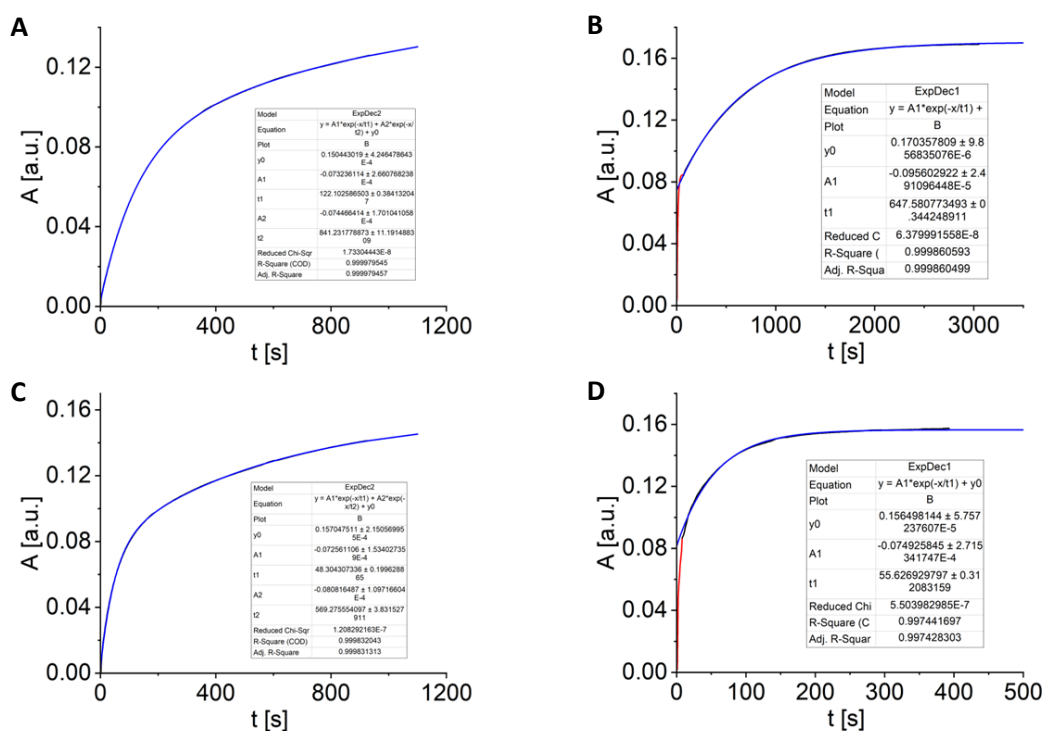


Figure S 95: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM IV-S_p/R_p by 300 nM PTE_I106A_F132A_S308A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of IV-S_p. **B** Time course for the hydrolysis of 10 μM IV-S_p/R_p by 500 nM PTE_I106A_F132A_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of IV-R_p. Data points corresponding to the hydrolysis of IV-S_p (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM IV-R_p/S_p by 50 nM PTE_I106A_F132A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of IV-R_p after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM IV-S_p/R_p by 500 nM PTE_I106A_F132A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of IV-S_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of IV-R_p (red) were not considered in the fit.

Substrate V

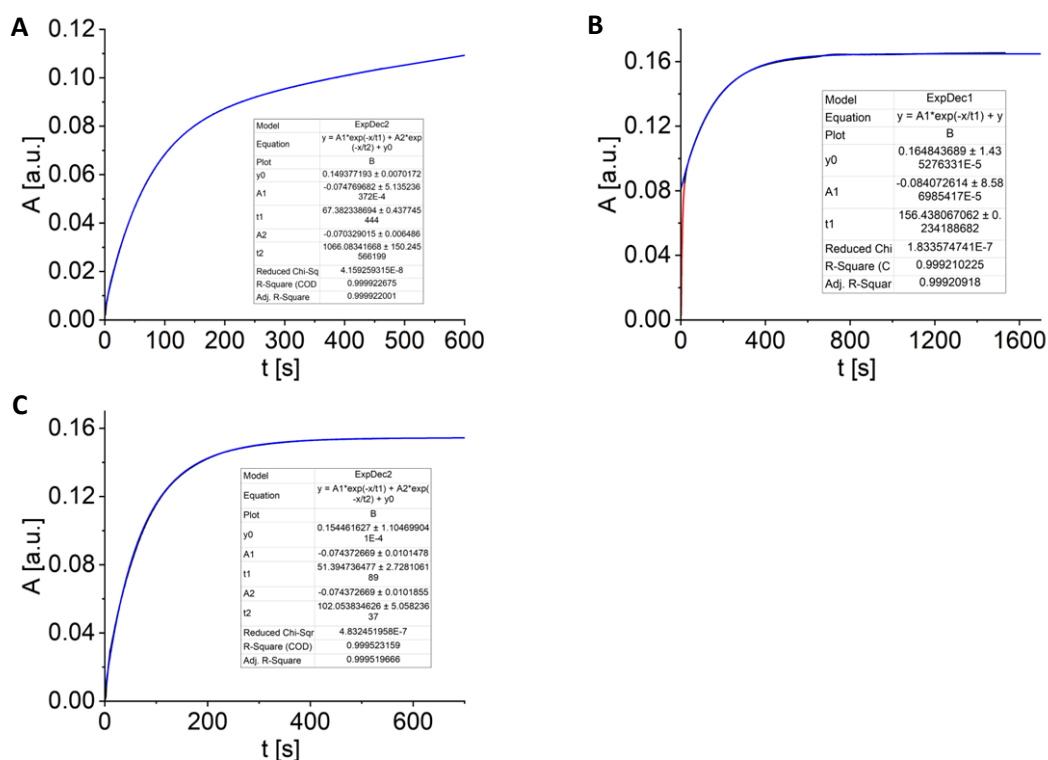


Figure S 96: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM V-S_P/R_P by 100 nM PTE_I106A_F132A_S308A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of V-S_P. **B** Time course for the hydrolysis of 10 μM V-S_P/R_P by 1 μM PTE_I106A_F132A_S308A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of V-R_P. Data points corresponding to the hydrolysis of V-S_P (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM V-S_P/R_P by 50 nM PTE_I106A_F132A_S308A_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_S308A_H257ONBY for the hydrolysis of V-R_P/S_P after irradiation at 365 nm.

1.1.21 PTE_I106A_F132A_H257Y_S308A

Substrate I

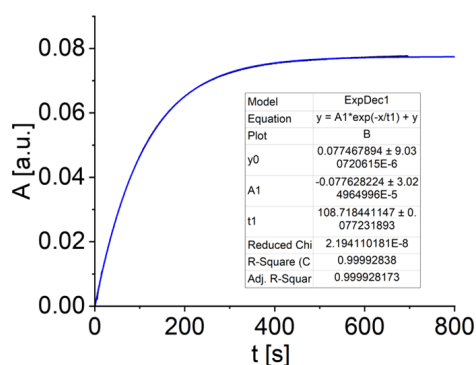


Figure S 97: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_F132A_H257Y_S308A

Time course for the hydrolysis of 5 μM substrate I by addition of 3 nM PTE_I106A_F132A_H257Y_S308A (black). The data was fitted to a single exponential function.

Substrate II

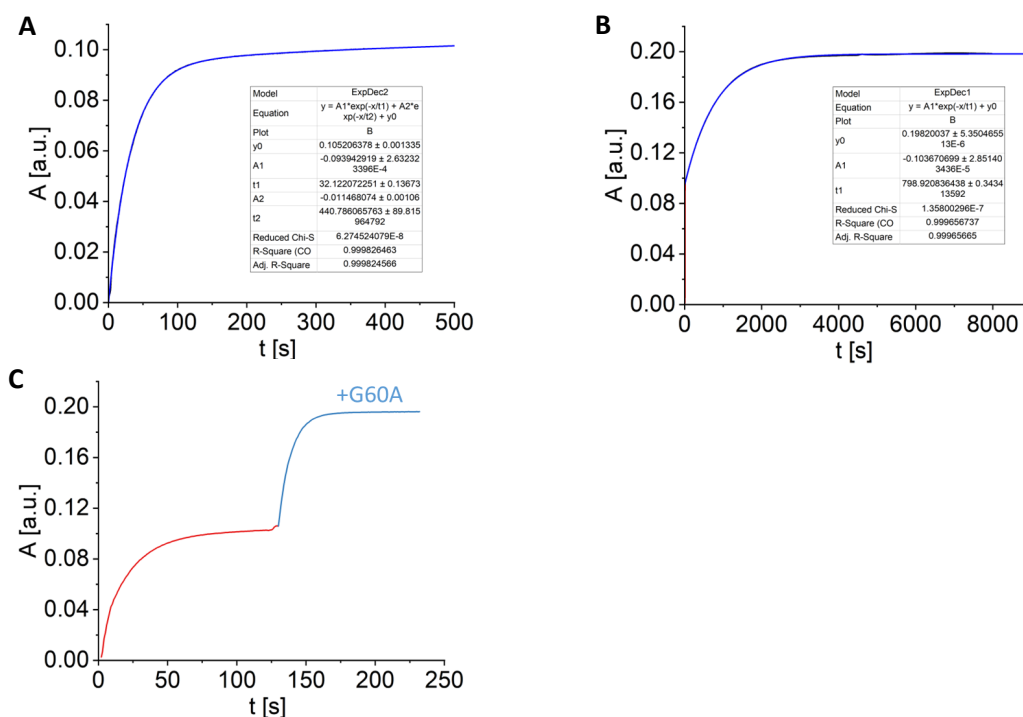


Figure S 98: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F132A_H257Y_S308A

A Time course for the hydrolysis of 10 μ M II- R_p/S_p by 1 nM PTE_I106A_F132A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y_S308A for the hydrolysis of II- R_p . **B** Time course for the hydrolysis of 10 μ M II- R_p/S_p by 10 nM PTE_I106A_F132A_H257Y_S308A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y_S308A for the hydrolysis of II- S_p . Data points corresponding to the hydrolysis of II- R_p (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H257Y_S308A. Enzymatic hydrolysis of 10 μ M substrate II was initiated by addition of 1 nM PTE_I106A_F132A_H257Y_S308A and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorption upon addition of PTE_G60A indicates that II- R_p is preferentially hydrolyzed.

Substrate III

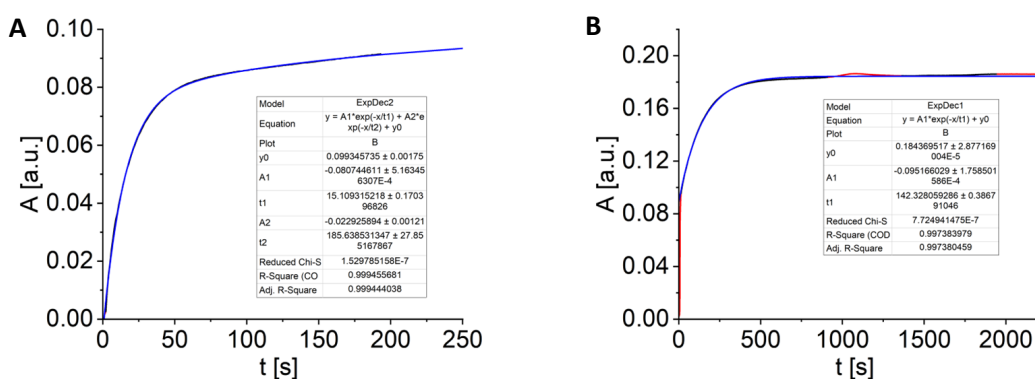


Figure S 99: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_H257Y_S308A

A Time course for the hydrolysis of 10 μ M III- S_p/R_p by 1 nM PTE_I106A_F132A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y_S308A for the hydrolysis of III- R_p . **B** Time course for the hydrolysis of 10 μ M III- R_p/S_p by 10 nM PTE_I106A_F132A_H257Y_S308A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y_S308A for the hydrolysis of III- S_p . Data points corresponding to the hydrolysis of III- R_p (red) were not considered in the fit.

Substrate IV

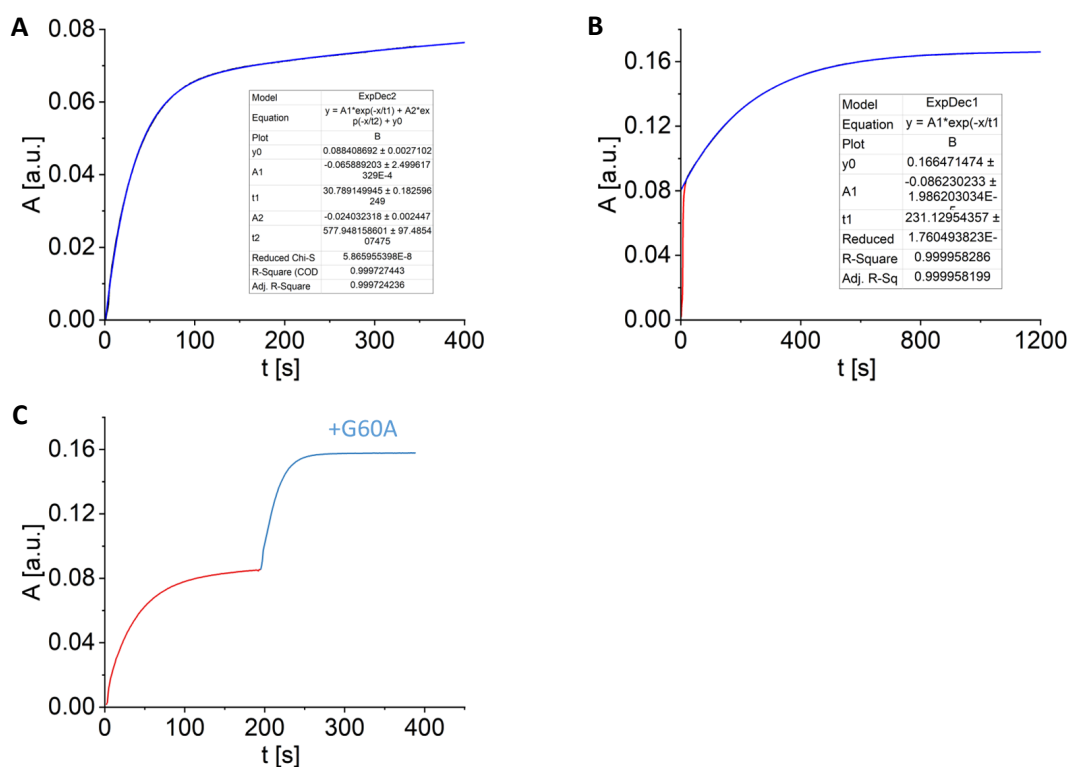


Figure S 100: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_H257Y_S308A

A Time course for the hydrolysis of 10 μ M IV-R_P/S_P by 50 nM PTE_I106A_F132A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y_S308A for the hydrolysis of IV-R_P. **B** Time course for the hydrolysis of 10 μ M IV-R_P/S_P by 500 nM PTE_I106A_F132A_H257Y_S308A (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y_S308A for the hydrolysis of IV-S_P. Data points corresponding to the hydrolysis of IV-R_P (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_I106A_F132A_H257Y_S308A. Enzymatic hydrolysis of 10 μ M substrate IV was initiated by addition of 50 nM PTE_I106A_F132A_H257Y_S308A and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in upon addition of PTE_G60A indicates that IV-R_P is preferentially hydrolyzed.

Substrate V

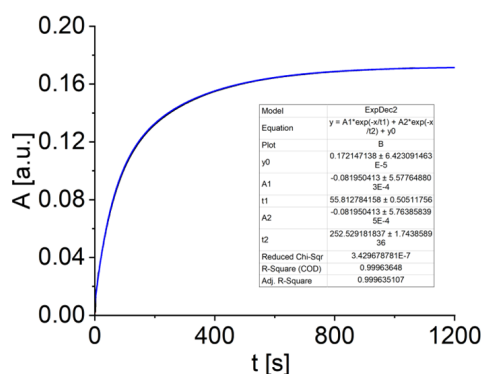


Figure S 101: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_H257Y_S308A

A Time course for the hydrolysis of 10 μ M V- R_P/S_P by 50 nM PTE_I106A_F132A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y_S308A for the hydrolysis of V-R_P/S_P.

1.1.22 PTE_H254G_L303T_H257ONBY

Substrate I

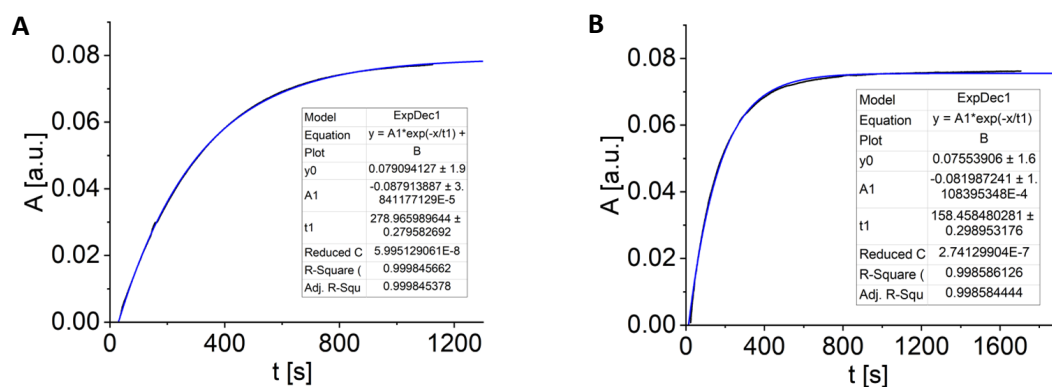


Figure S 102: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254G_L303T_H257ONBY before and after illumination

A Time course for the hydrolysis of 5 μM substrate I by 20 nM PTE_H254G_L303T_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue). **B** Time course for the hydrolysis of 5 μM substrate I by 5 nM PTE_H254G_L303T_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue).

Substrate II

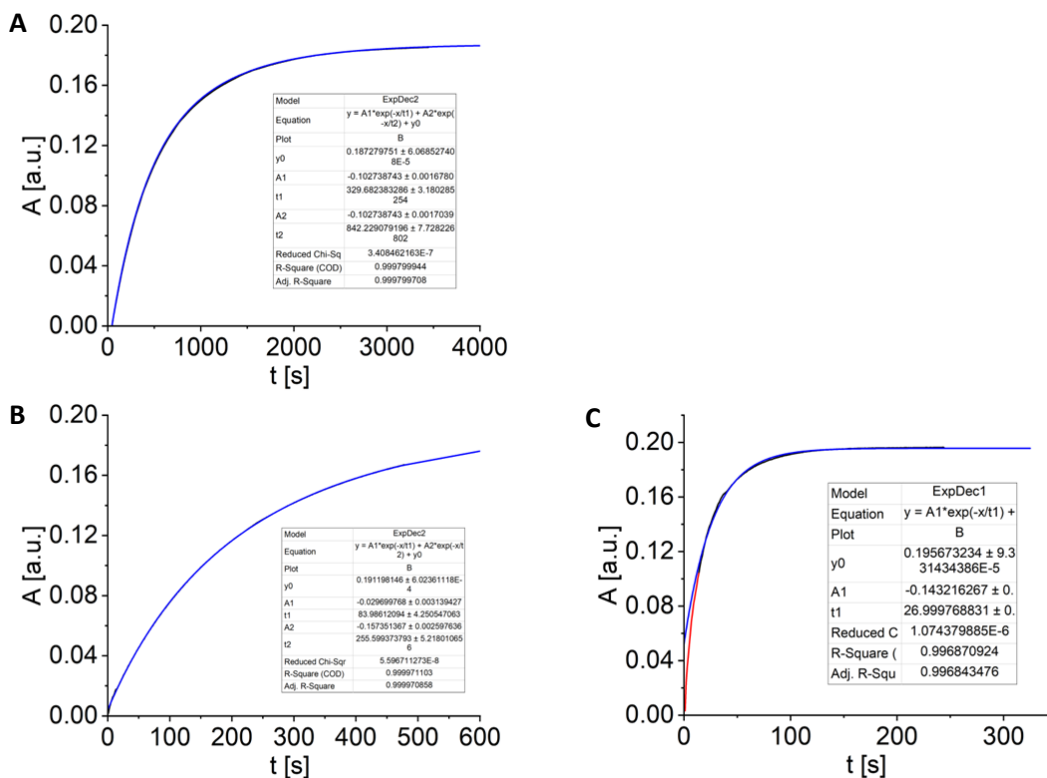


Figure S 103: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254G_L303T_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM II-S_P/R_P by 5 nM PTE_H254G_L303T_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of II-S_P/R_P. **B** Time course for the hydrolysis of 10 μM II-R_P/S_P by 1 nM PTE_H254G_L303T_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of II-R_P after irradiation at 365 nm. **C** Time course for the hydrolysis of 10 μM II-R_P/S_P by 10 nM PTE_H254G_L303T_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of II-S_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of II-R_P (red) were not considered in the fit.

Substrate III

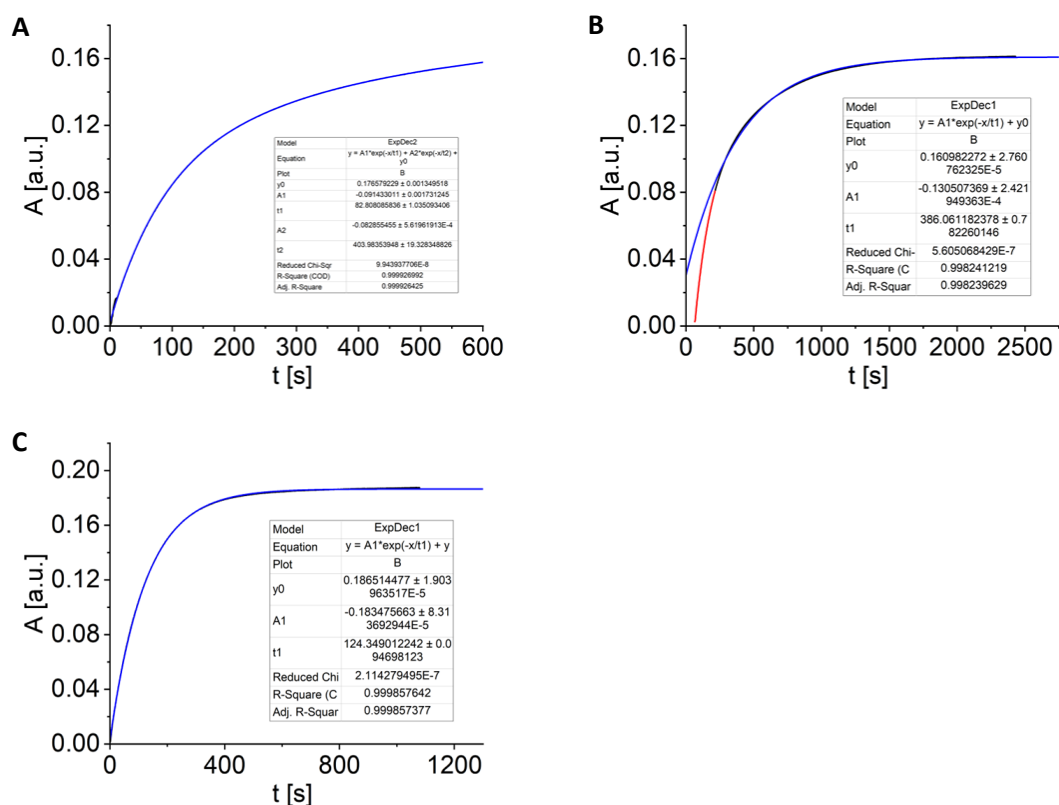


Figure S 104: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254G_L303T_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM III-R_P/S_P by 5 nM PTE_H254G_L303T_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of III-S_P. **B** Time course for the hydrolysis of 10 μM III-R_P/S_P by 10 nM PTE_H254G_L303T_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of III-R_P after irradiation at 365 nm. Data points corresponding to the hydrolysis of III-S_P (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM III-R_P/S_P by 5 nM PTE_H254G_L303T_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of III-S_P/R_P after irradiation at 365 nm.

Substrate IV

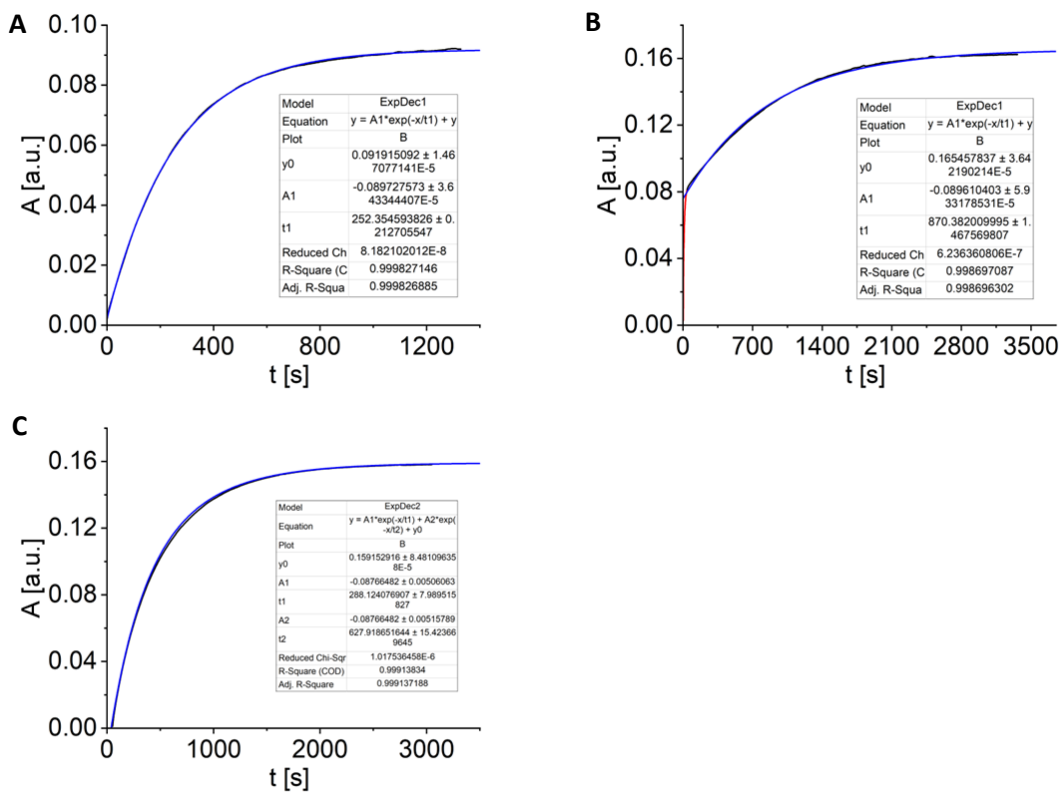


Figure S 105: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254G_L303T_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μ M IV-S_P/R_P by 20 nM PTE_H254G_L303T_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of IV-S_P. **B** Time course for the hydrolysis of 10 μ M IV-S_P/R_P by 500 nM PTE_H254G_L303T_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of IV-R_P. Data points corresponding to the hydrolysis of IV-S_P (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μ M IV-S_P/R_P by 20 nM PTE_H254G_L303T_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of IV-S_P/R_P after irradiation at 365 nm.

Substrate V

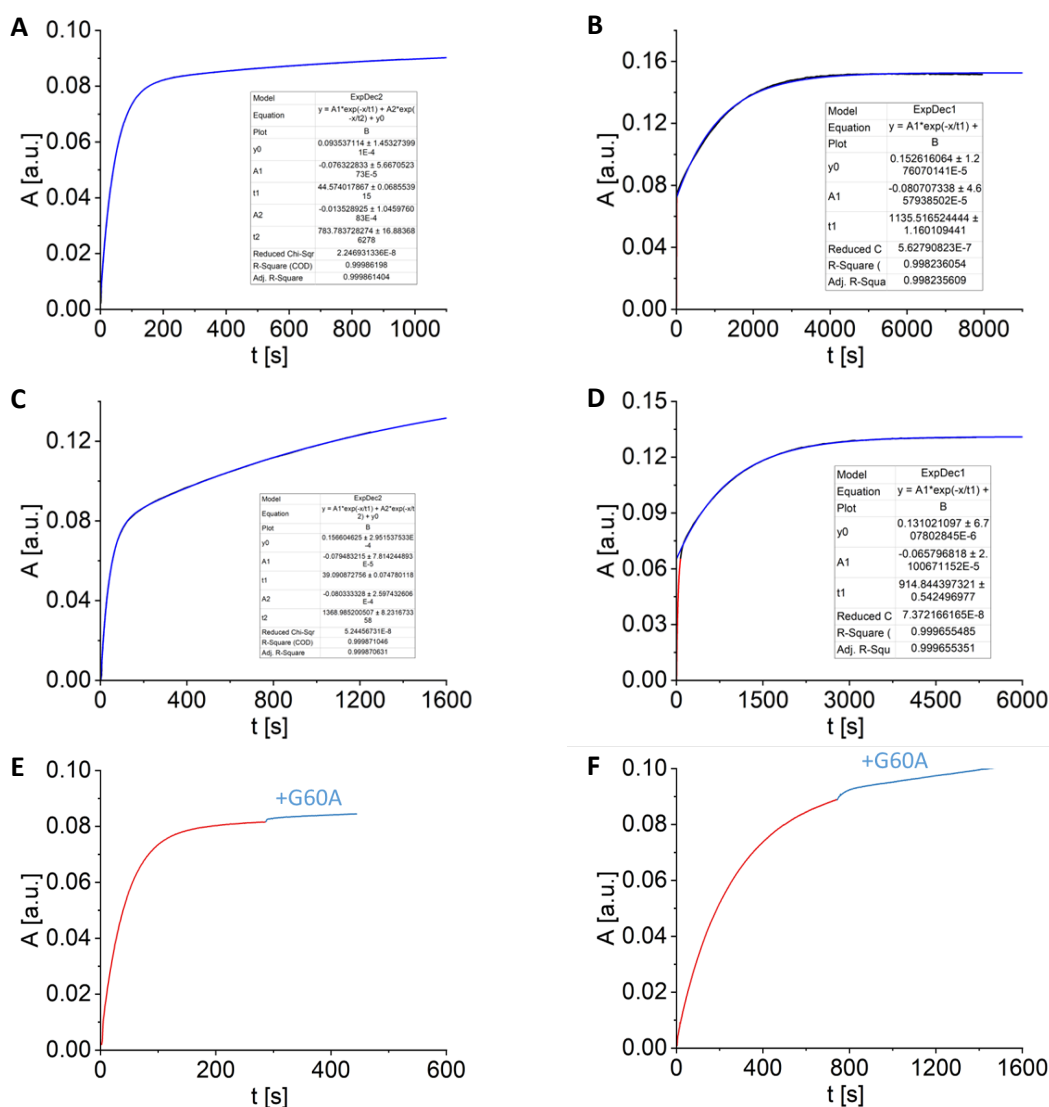


Figure S 106: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254G_L303T_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 μM V-S_p/R_p by 40 nM PTE_H254G_L303T_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of V-S_p. **B** Time course for the hydrolysis of 10 μM V-S_p/R_p by 1 μM PTE_H254G_L303T_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of V-R_p. Data points corresponding to the hydrolysis of V-S_p (red) were not considered in the fit. **C** Time course for the hydrolysis of 10 μM V-S_p/R_p by 20 nM PTE_H254G_L303T_H257ONBY after irradiation at 365 nm (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of V-S_p after irradiation at 365 nm. **D** Time course for the hydrolysis of 10 μM V-S_p/R_p by 50 nM PTE_H254G_L303T_H257ONBY after irradiation at 365 nm (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_L303T_H257ONBY for the hydrolysis of V-R_p after irradiation at 365 nm. Data points corresponding to the hydrolysis of V-S_p (red) were not considered in the fit. **E** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_H254G_L303T_H257ONBY *as isolated*. Enzymatic hydrolysis of 10 μM substrate V was initiated by addition of 40 nM PTE_H254G_L303T_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that IV-S_p is preferentially hydrolyzed. **F** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_H254G_L303T_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 μM substrate V was initiated by addition of 10 nM PTE_H254G_L303T_H257ONBY and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected upon addition of PTE_G60A, implying that V-S_p is preferentially hydrolyzed and thus, there is no inversion of stereoselectivity after irradiation at 365 nm.

1.1.23 PTE_H254G_H257Y_L303T

Substrate I

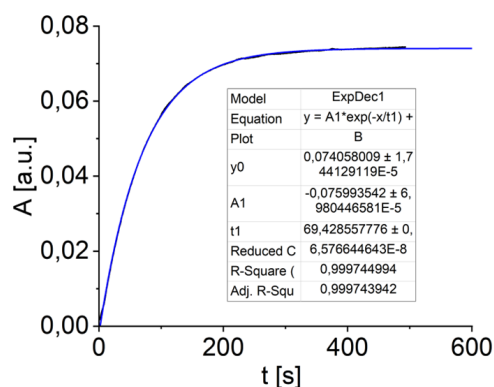


Figure S 107: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254G_H257Y_L303T

Time course for the hydrolysis of 5 μM substrate I by addition of 5 nM PTE_H254G_H257Y_L303T (black). The data was fitted to a single exponential function.

Substrate II

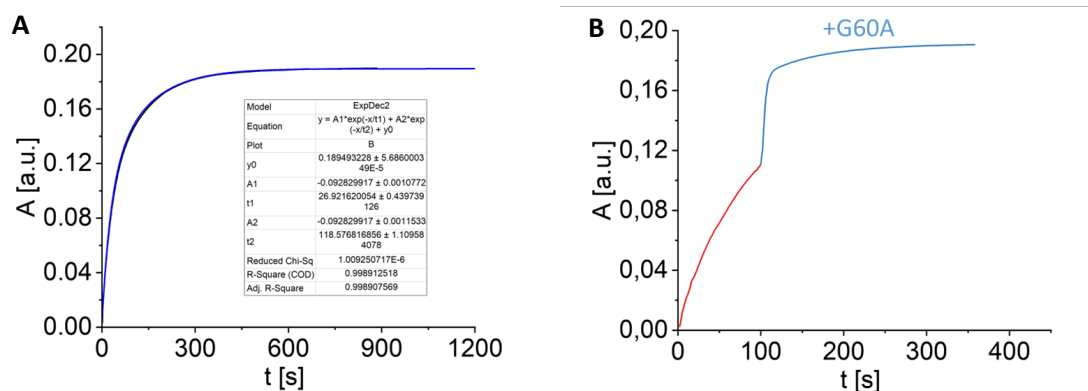


Figure S 108: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254G_H257Y_L303T

A Time course for the hydrolysis of 10 μM II-R_P/S_P by 5 nM PTE_H254G_H257Y_L303T (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_H257Y_L303T for the hydrolysis of II-R_P/S_P. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_H254G_H257Y_L303T. Enzymatic hydrolysis of 10 μM substrate II was initiated by addition of 2 nM PTE_H254G_H257Y_L303T and PTE_G60A (5 nM) was added after one isomer was almost quantitatively hydrolyzed. The noticeable increase in absorbance upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed.

Substrate III

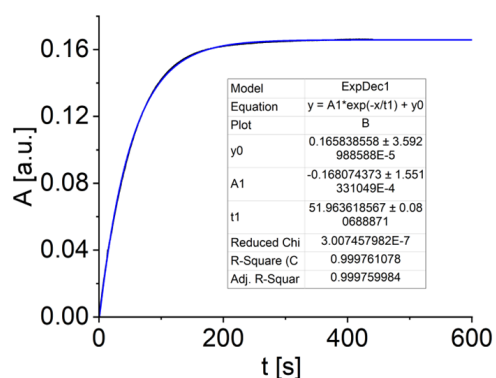


Figure S 109: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254G_H257Y_L303T

A Time course for the hydrolysis of 10 μM III-S_P/R_P by 5 nM PTE_H254G_H257Y_L303T (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_H257Y_L303T for the hydrolysis of III-R_P/S_P.

Substrate IV

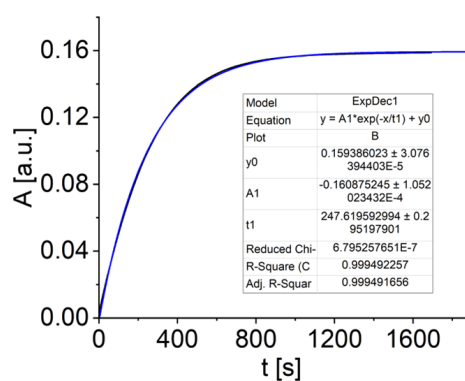


Figure S 110: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254G_H257Y_L303T

A Time course for the hydrolysis of 10 μM IV-R_P/S_P by 20 nM PTE_H254G_H257Y_L303T (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_H257Y_L303T for the hydrolysis of IV-S_P/R_P.

Substrate V

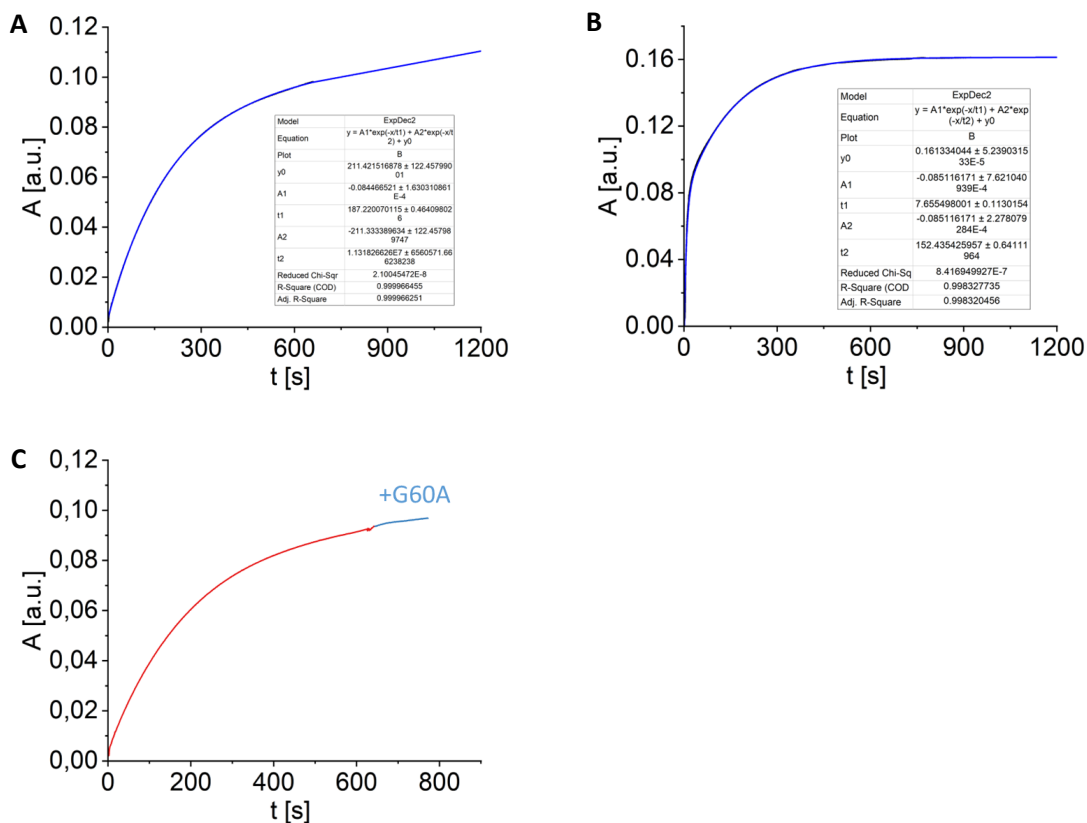


Figure S 111: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254G_H257Y_L303T

A Time course for the hydrolysis of 10 μM V-S_p/R_p by 10 nM PTE_H254G_H257Y_L303T (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_H257Y_L303T for the hydrolysis of V-S_p. **B** Time course for the hydrolysis of 10 μM V-S_p/R_p by 200 nM PTE_H254G_H257Y_L303T (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_H257Y_L303T for the hydrolysis of V-R_p. Data points corresponding to the hydrolysis of V-S_p (red) were not considered in the fit. **C** Complementation assay with PTE_G60A to identify the enantiomer preferred by PTE_H254G_H257Y_L303T. Enzymatic hydrolysis of 10 μM substrate V was initiated by addition of 10 nM PTE_H254G_H257Y_L303T and PTE_G60A (15 nM) was added after one isomer was almost quantitatively hydrolyzed. The curve is mostly unaffected by addition of PTE_G60A, implying that V-S_p is preferentially hydrolyzed.