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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1.1 Time Course of Substrate Hydrolysis

1.1.1 Chemical Hydrolysis

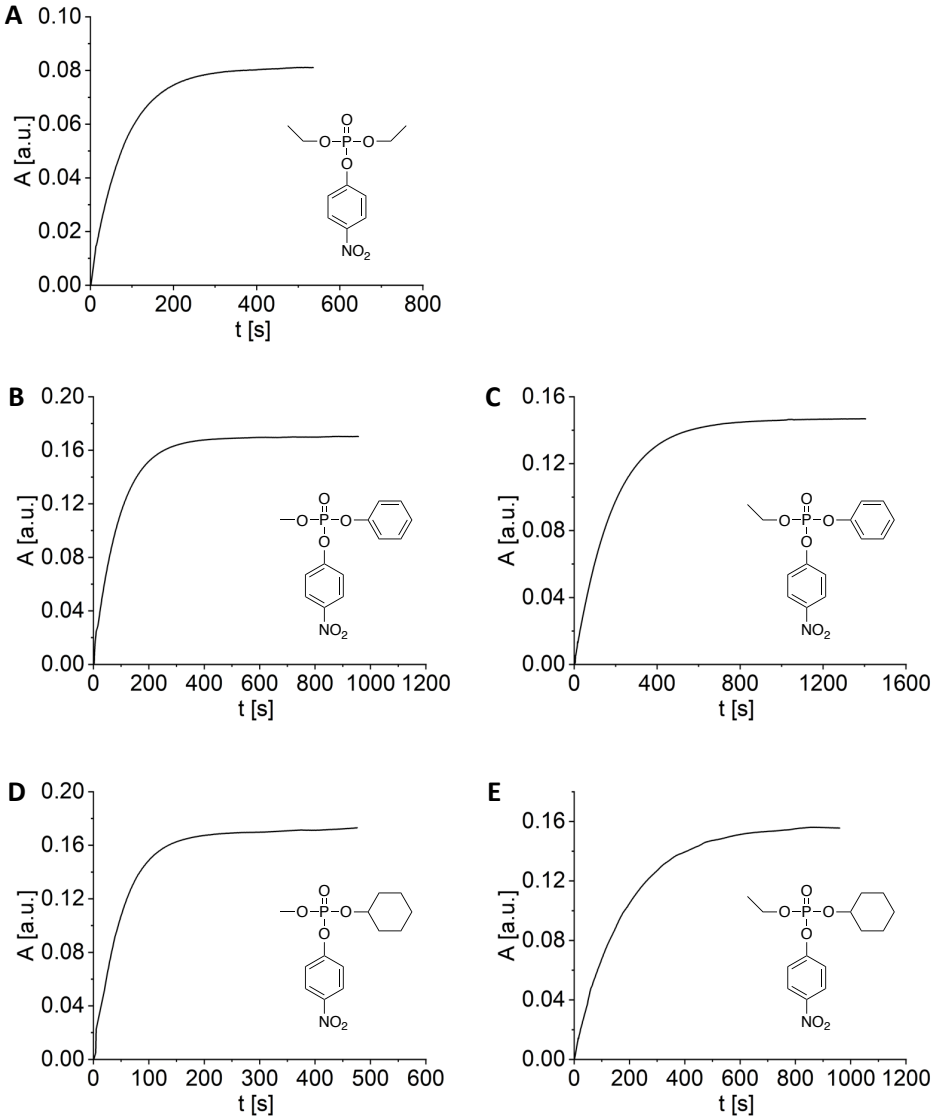

Figure S 1: Chemical hydrolysis of PTE substrates used in this work

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

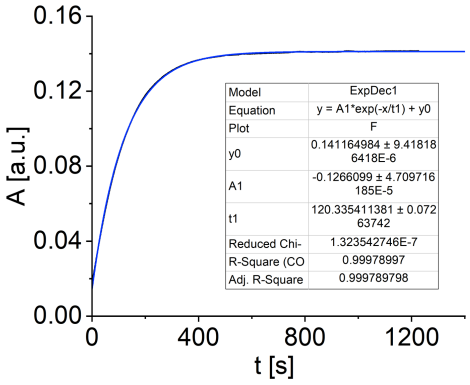

Figure S 2: Enzymatic hydrolysis of substrate I catalyzed by PTE_wt
Time course for the hydrolysis of 10 µM substrate I by addition of 0.1 nM PTE_wt (black). The data was fitted to a single exponential function (blue).

Substrate II

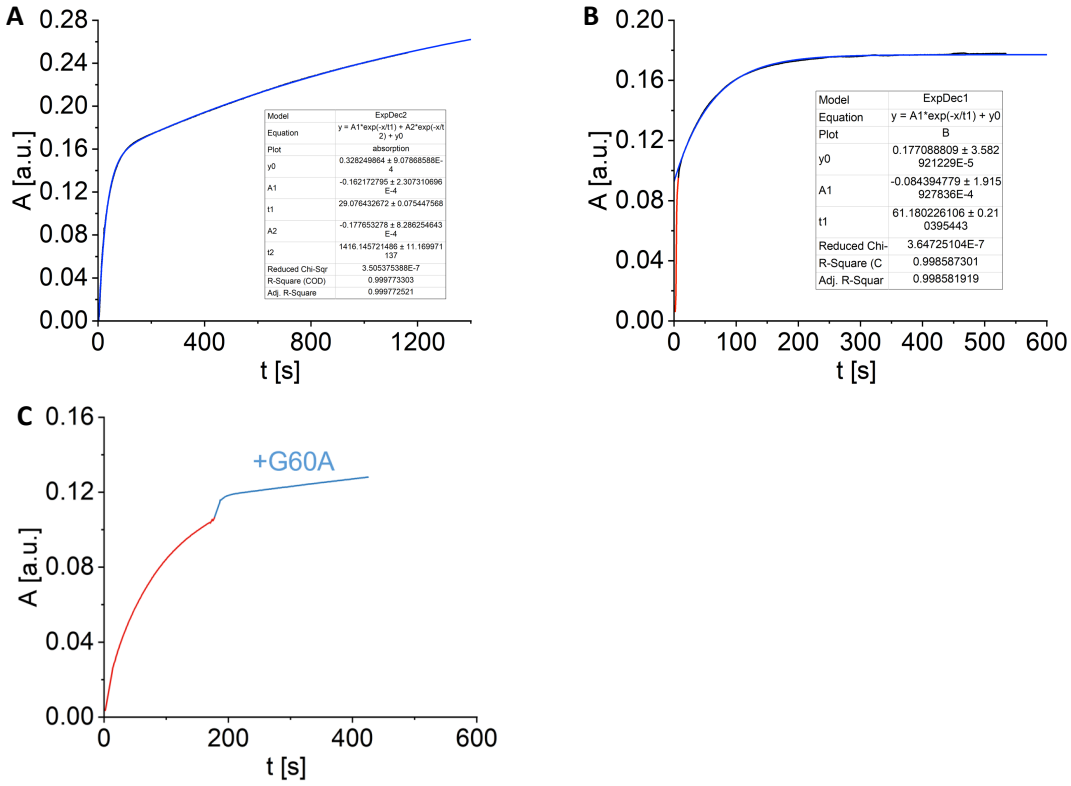

Figure S 3: Enzymatic hydrolysis of substrate II catalyzed by PTE_wt
A Time course for the hydrolysis of 20 µM II-S_P/R_P by 0.5 nM PTE_wt (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_wt for the hydrolysis of II-S_P. **B** 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

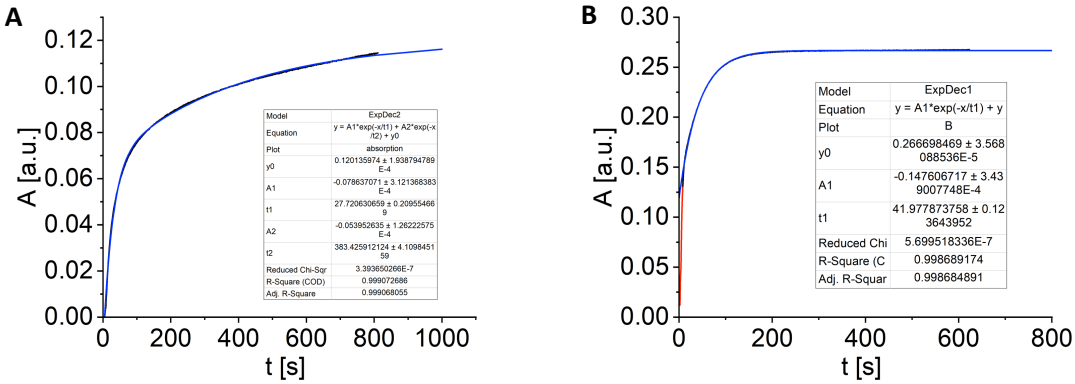

Figure S 4: Enzymatic hydrolysis of substrate III catalyzed by PTE_wt
A Time course for the hydrolysis of 20 µM III-S_P/R_P by 0.5 nM PTE_wt (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_wt for the hydrolysis of III-S_P. **B** Time course for the hydrolysis of 20 µM 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

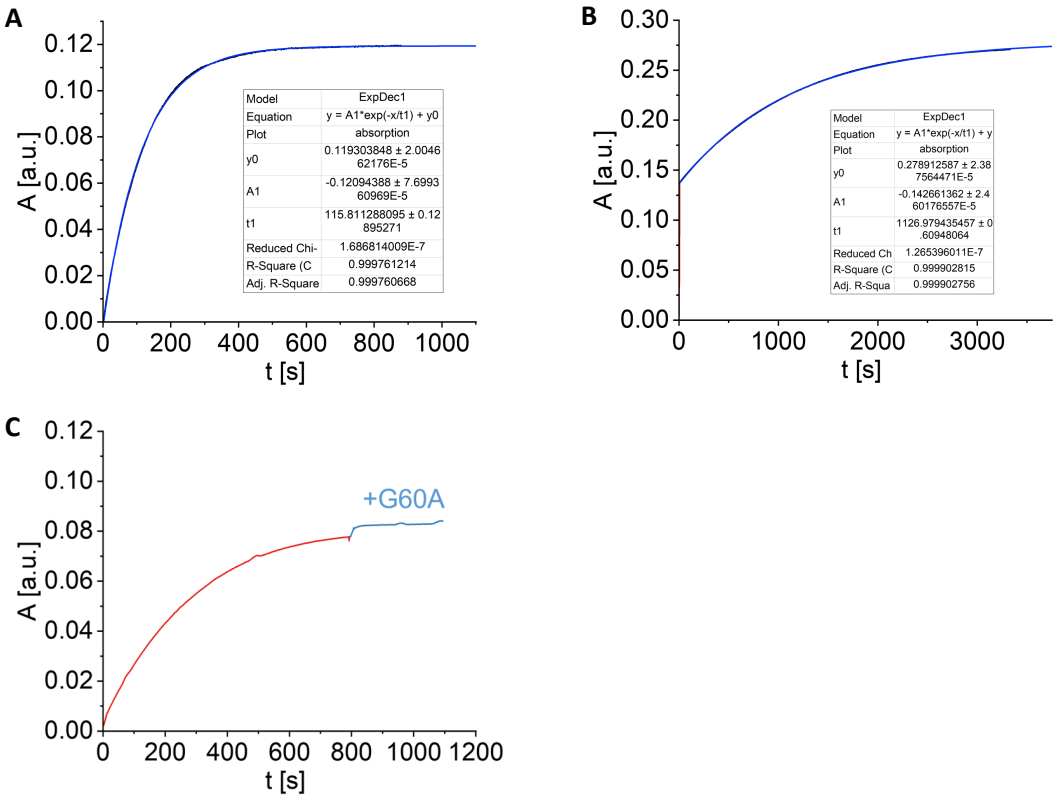

Figure S 5: Enzymatic hydrolysis of substrate IV catalyzed by PTE_wt

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

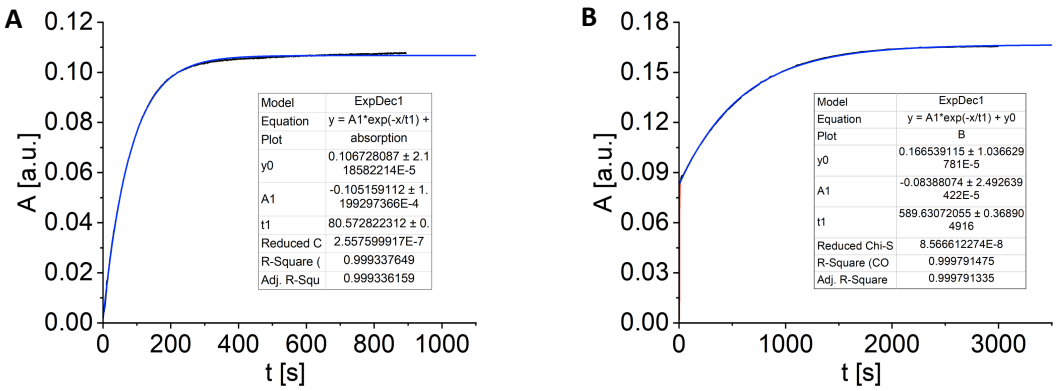

Figure S 6: Enzymatic hydrolysis of substrate V catalyzed by PTE_wt

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

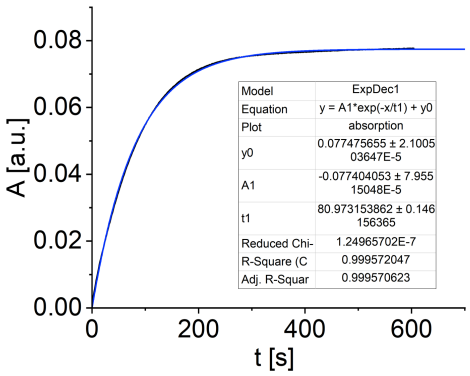

Figure S 7 Enzymatic hydrolysis of substrate I catalyzed by PTE_G60A
Time course for the hydrolysis of 5 µM substrate I by addition of 2 nM PTE_G60A (black). The data was fitted to a single exponential function.

Substrate II

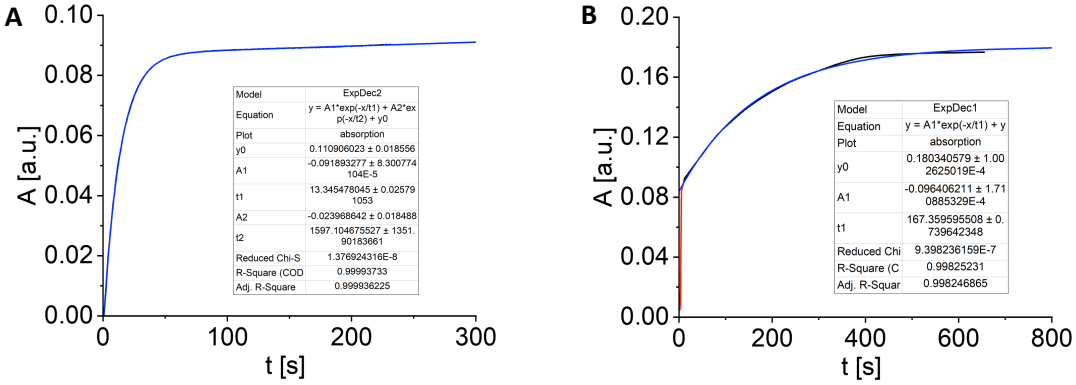

Figure S 8: Enzymatic hydrolysis of substrate II catalyzed by PTE_G60A
A Time course for the hydrolysis of 10 µM II-S_P/R_P by 2 nM PTE_G60A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_G60A for the hydrolysis of II-S_P. **B** Time course for the hydrolysis of 10 µM II-S_P/R_P by 1 µM 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

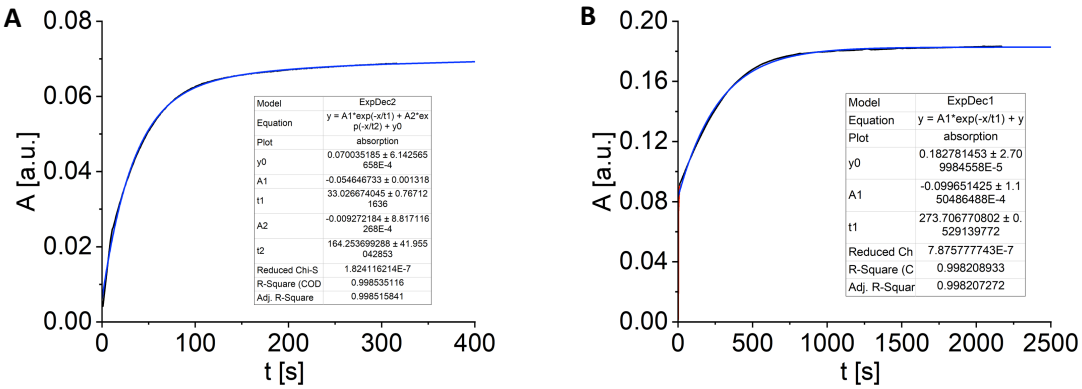

Figure S 9: Enzymatic hydrolysis of substrate III catalyzed by PTE_G60A

A Time course for the hydrolysis of 10 µM 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 µM III-S_P/R_P by 1 µM 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

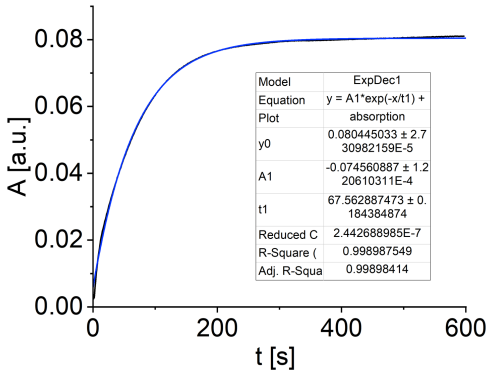

Figure S 10: Enzymatic hydrolysis of substrate IV catalyzed by PTE_G60A

A Time course for the hydrolysis of 10 µM 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

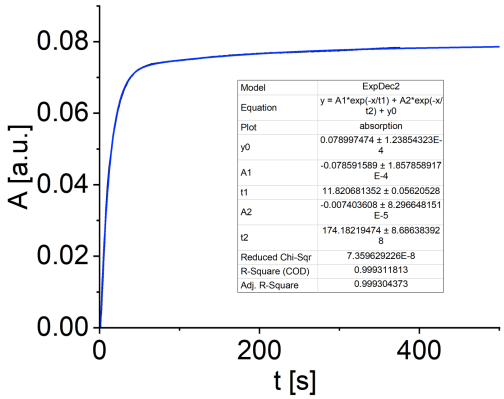

Figure S 11: Enzymatic hydrolysis of substrate V catalyzed by PTE_G60A
A Time course for the hydrolysis of 20 µM V-S_P/R_P by 5 nM PTE_G60A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_G60A for the hydrolysis of V-S_P.

1.1.4 PTE_H254ONBY

Substrate I

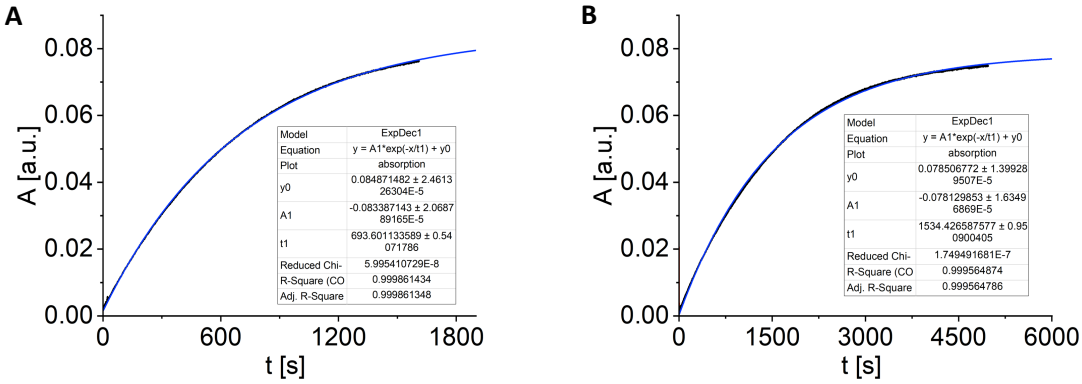

Figure S 12: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254ONBY before and after illumination
A Time course for the hydrolysis of 5 µM substrate I by 1 nM PTE_H254ONBY *as isolated* (black). The data was fitted to a single exponential function (blue). **B** Time course for the hydrolysis of 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

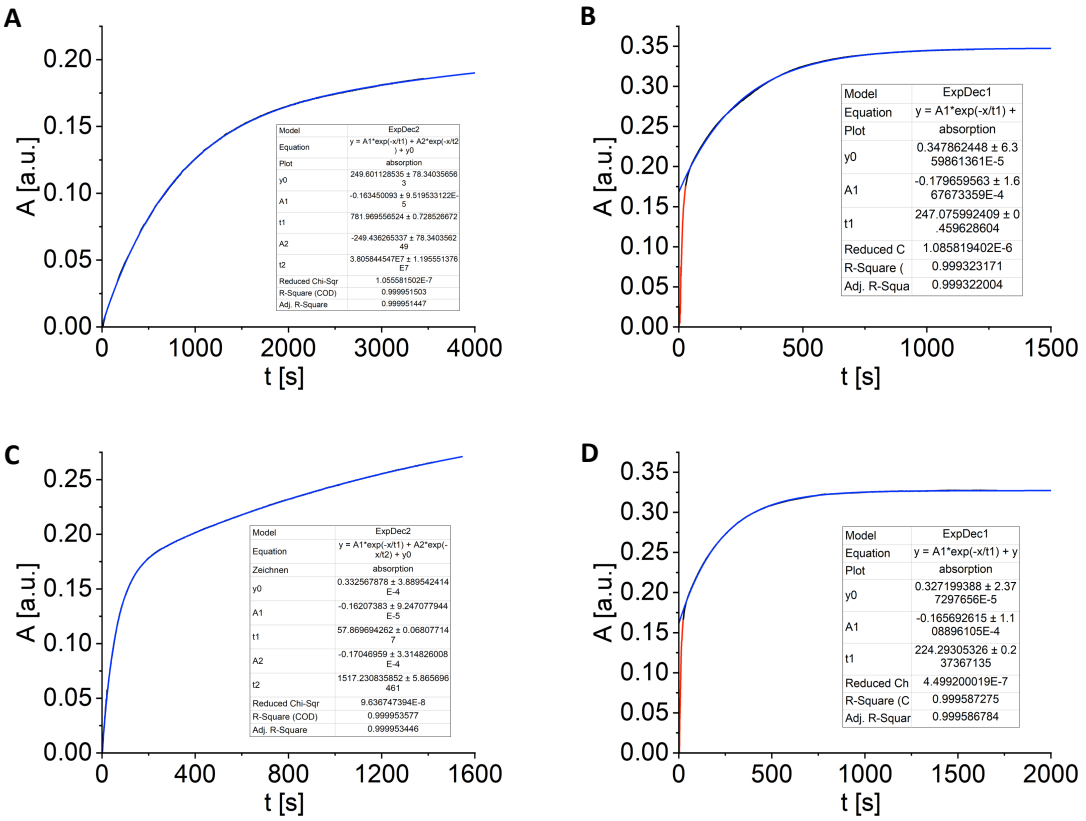

Figure S 13: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254ONBY before and after illumination

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

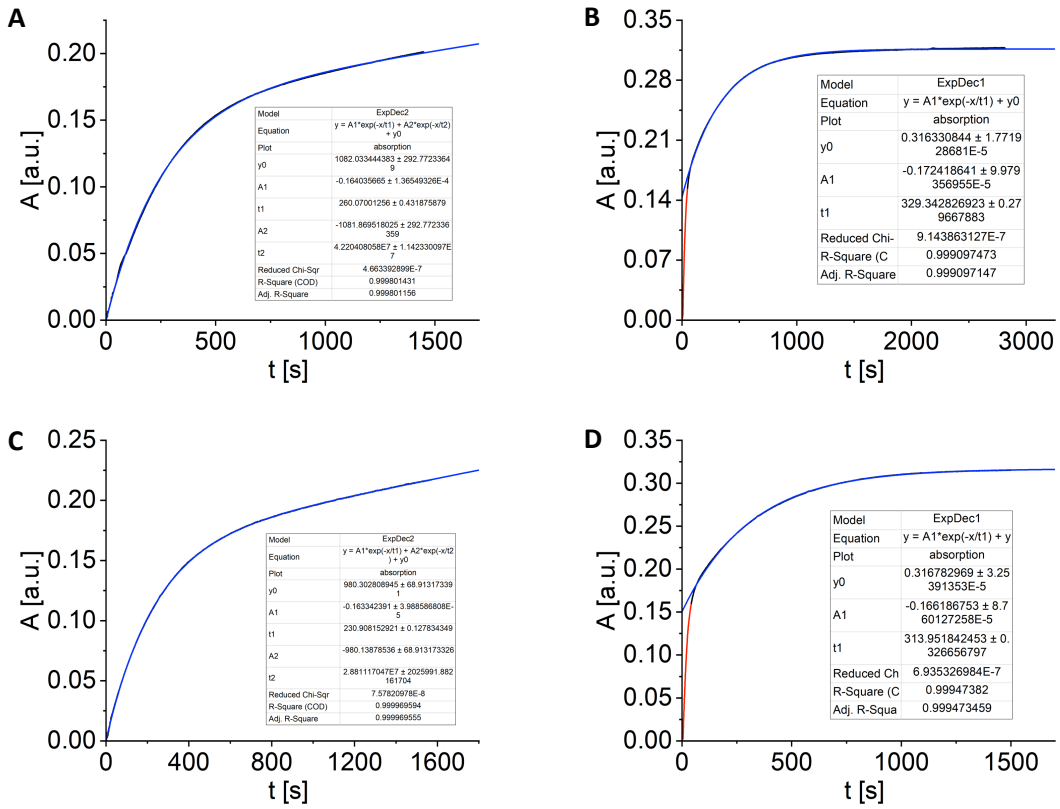

Figure S 14: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254ONBY before and after illumination

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

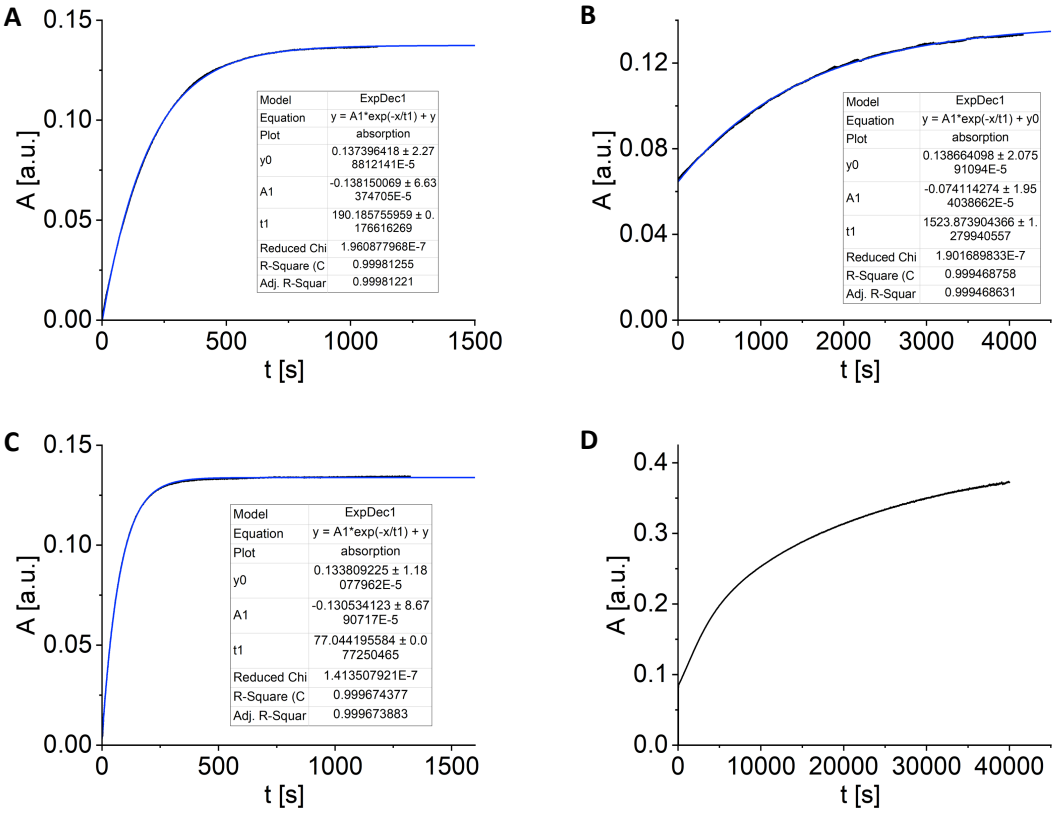

Figure S 15: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254ONBY before and after illumination
A Time course for the hydrolysis of 20 µM IV-S_P/R_P by 10 nM PTE_H254ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic 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

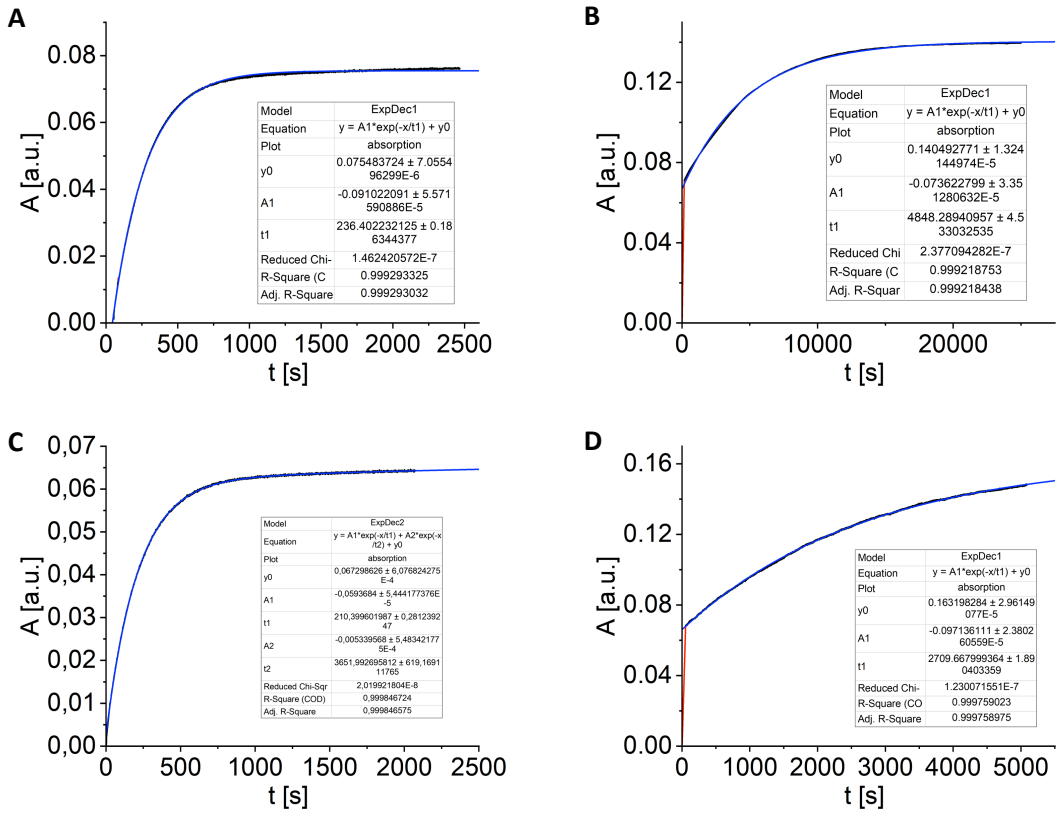

Figure S 16: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254ONBY before and after illumination

A Time course for the hydrolysis of 5 µM 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 µM V-S_P/R_P by 5 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 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 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 µM V-S_P/R_P by 15 µM 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-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

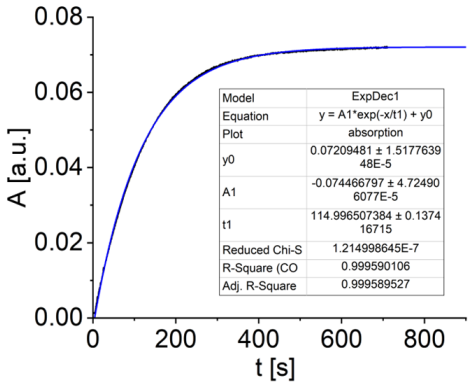

Figure S 17: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254Y #1
Time course for the hydrolysis of 5 µM substrate I by addition of 5 nM PTE_H254Y #1 (black). The data was fitted to a single exponential function.

Substrate II

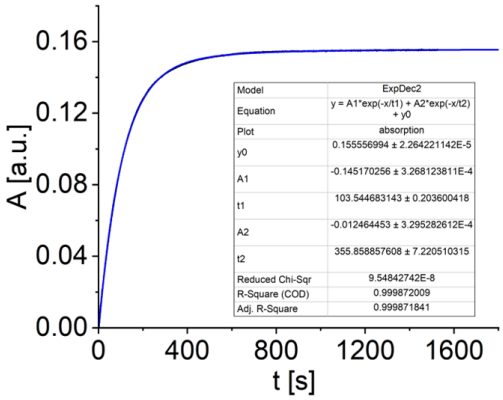

Figure S 18: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254Y #1
A Time course for the hydrolysis of 10 µM II-S_P/R_P by 200 nM PTE_H254Y #1 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #1 for the hydrolysis of II-S_P/R_P.

Substrate III

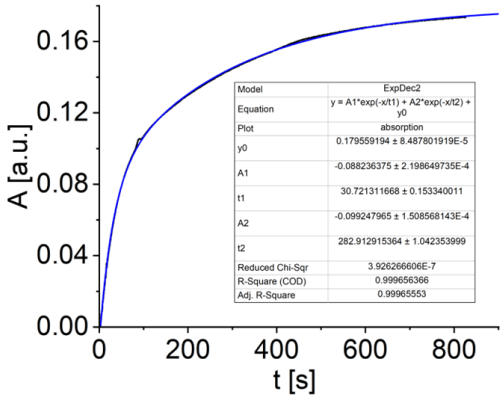

Figure S 19: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254Y #1
A Time course for the hydrolysis of 10 µM III-S_P/R_P by 200 nM PTE_H254Y #1 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #1 for the hydrolysis of III-S_P/III-R_P.

Substrate IV

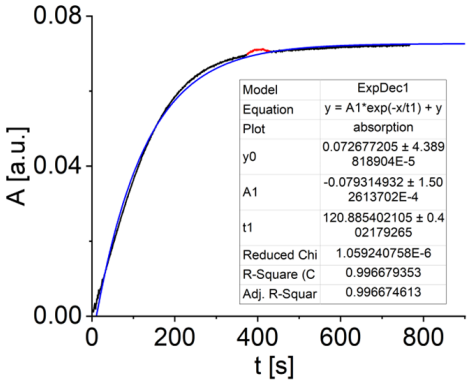

Figure S 20: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254Y #1
A Time course for the hydrolysis of 10 µM IV-S_P/R_P by 500 nM PTE_H254Y #1 (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #1 for the hydrolysis of IV-S_P.

Substrate V

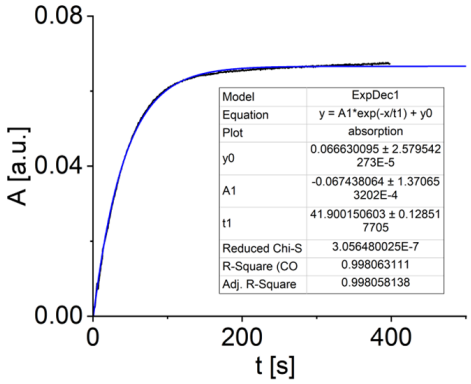

Figure S 21: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254Y #1
A Time course for the hydrolysis of 10 µM V-S_P/R_P by 50 nM PTE_H254Y #1 (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #1 for the hydrolysis of V-S_P.

1.1.6 PTE_H254Y #2

Substrate I

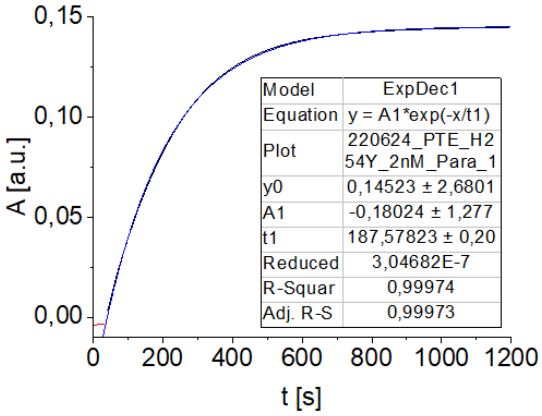

Figure S 22: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254Y #2
Time course for the hydrolysis of 10 µM substrate I by addition of 2 nM PTE_H254Y #2 (black). The data was fitted to a single exponential function.

Substrate II

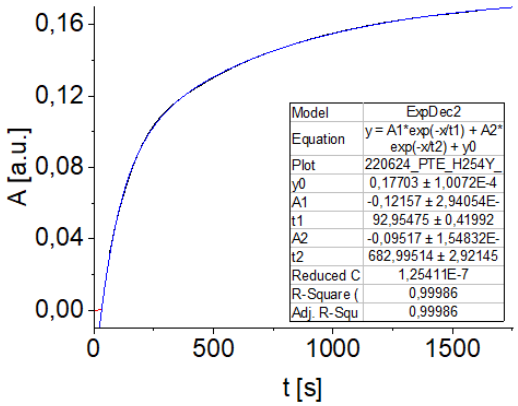

Figure S 23: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254Y #2

A Time course for the hydrolysis of 10 µM 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

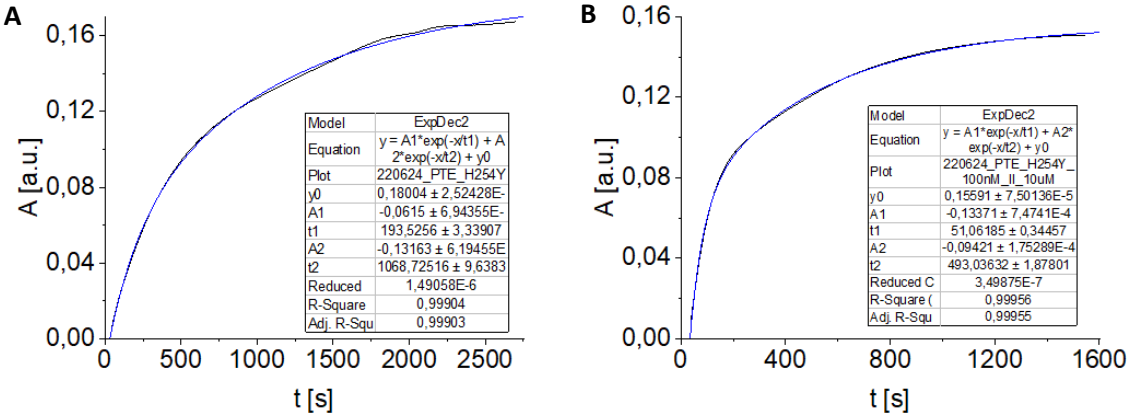

Figure S 24: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254Y #2

A Time course for the hydrolysis of 10 µM 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 µM 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

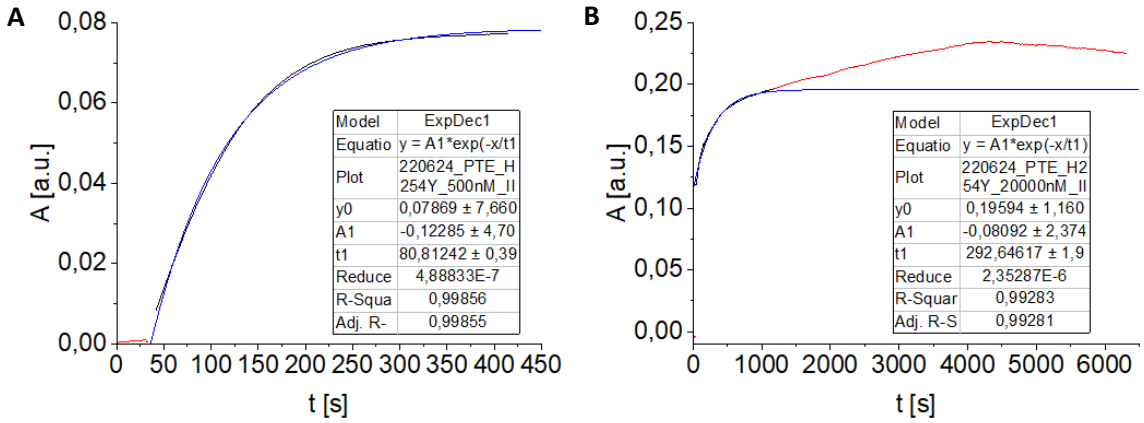

Figure S 25: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254Y #2

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

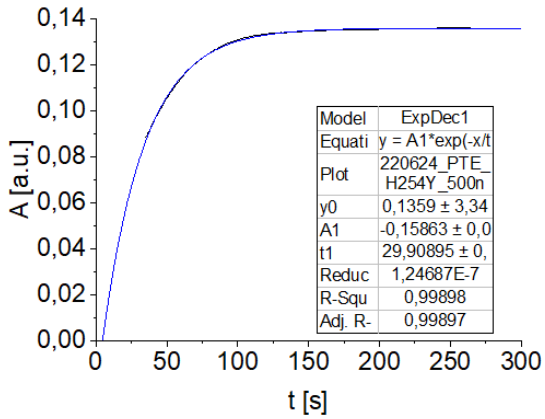

Figure S 26: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254Y #2

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

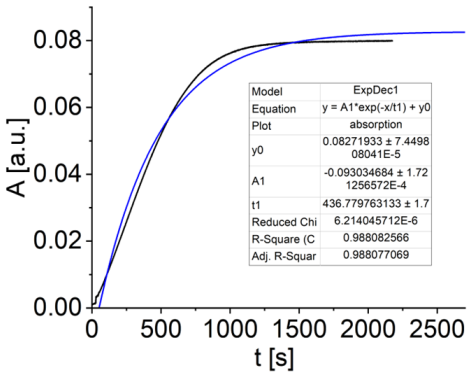

Figure S 27: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254Y #3
Time course for the hydrolysis of 5 µM substrate I by addition of 50 nM PTE_H254Y #3 (black). The data was fitted to a single exponential function.

Substrate II

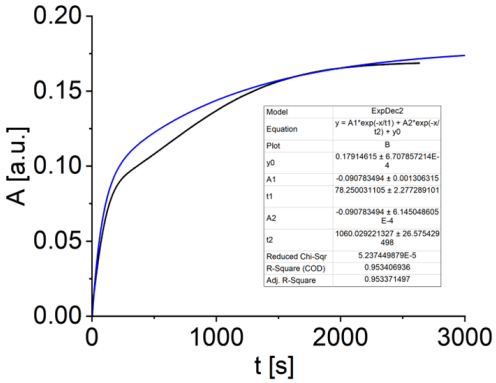

Figure S 28: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254Y #3
A Time course for the hydrolysis of 10 µM II-S_P/R_P by 200 nM PTE_H254Y #3 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #3 for the hydrolysis of II-S_P/R_P.

Substrate III

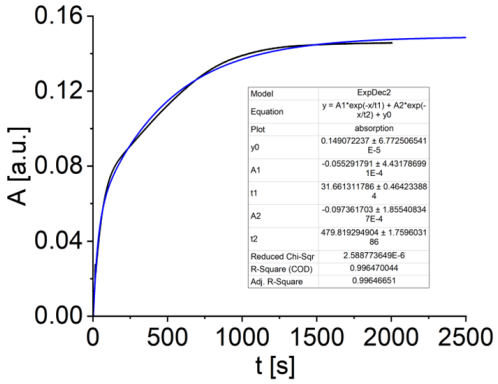

Figure S 29: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254Y #3
A Time course for the hydrolysis of 10 µM III-S_P/R_P by 200 nM PTE_H254Y #3 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #3 for the hydrolysis of III-S_P/R_P.

Substrate IV

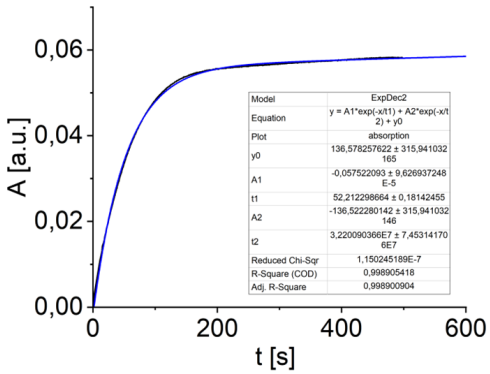

Figure S 30: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254Y #3
A Time course for the hydrolysis of 10 µM IV-S_P/R_P by 1 µM PTE_H254Y #3 (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254Y #3 for the hydrolysis of IV-S_P.

Substrate V

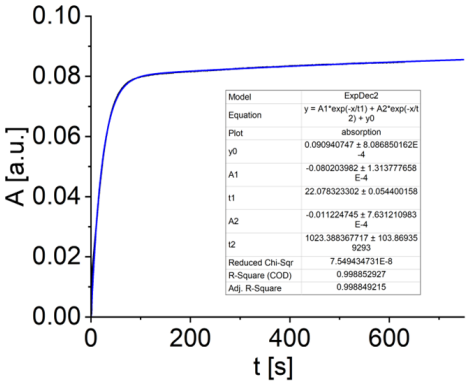

Figure S 31: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254Y #3

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

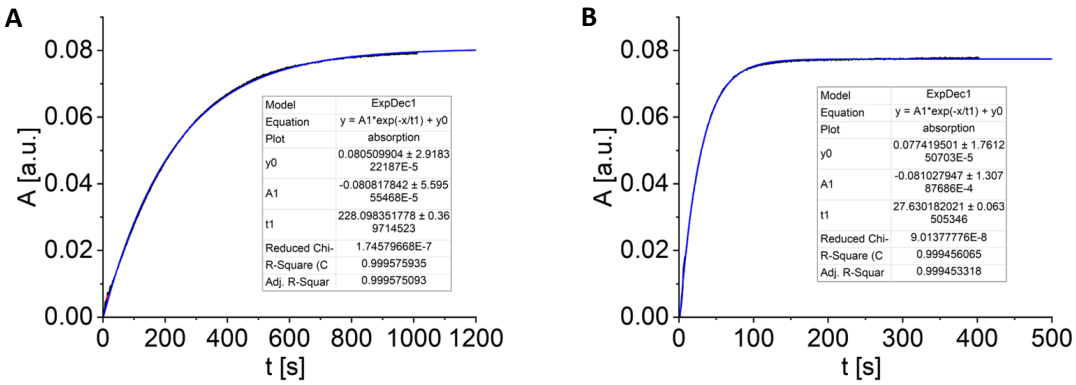

Figure S 32: Enzymatic hydrolysis of substrate I catalyzed by PTE_H257ONBY before and after illumination

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

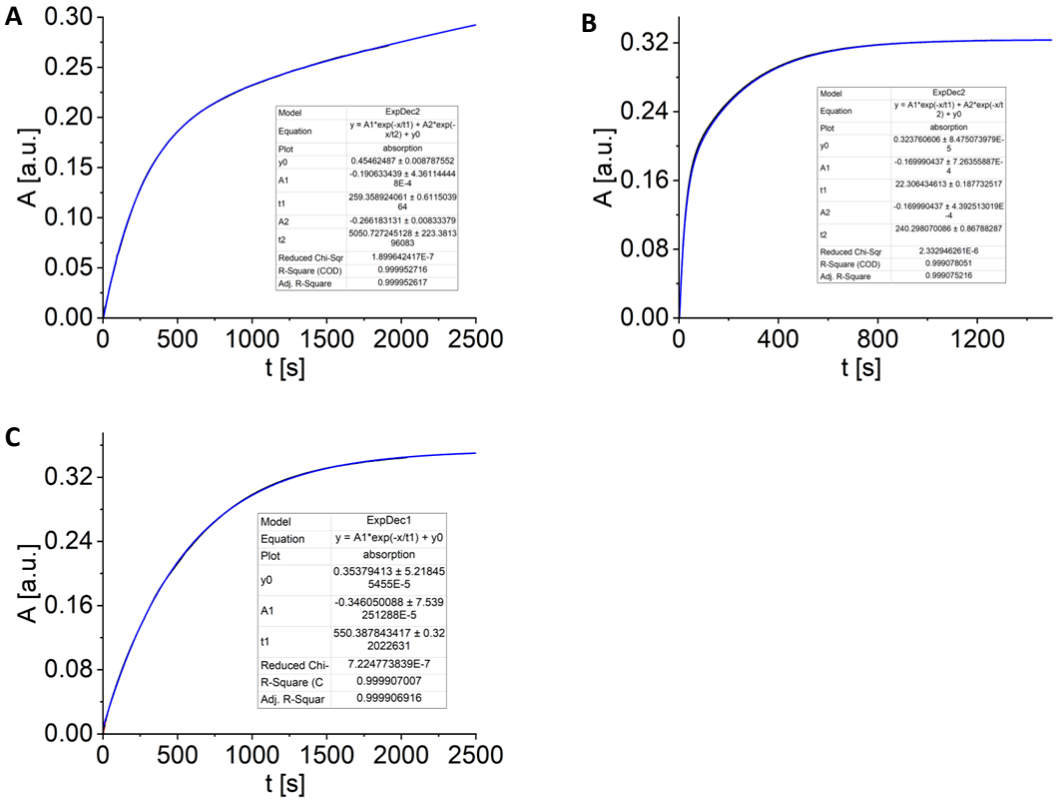

Figure S 33: Progress curve for PTE_H257ONBY catalyzed substrate II hydrolysis

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

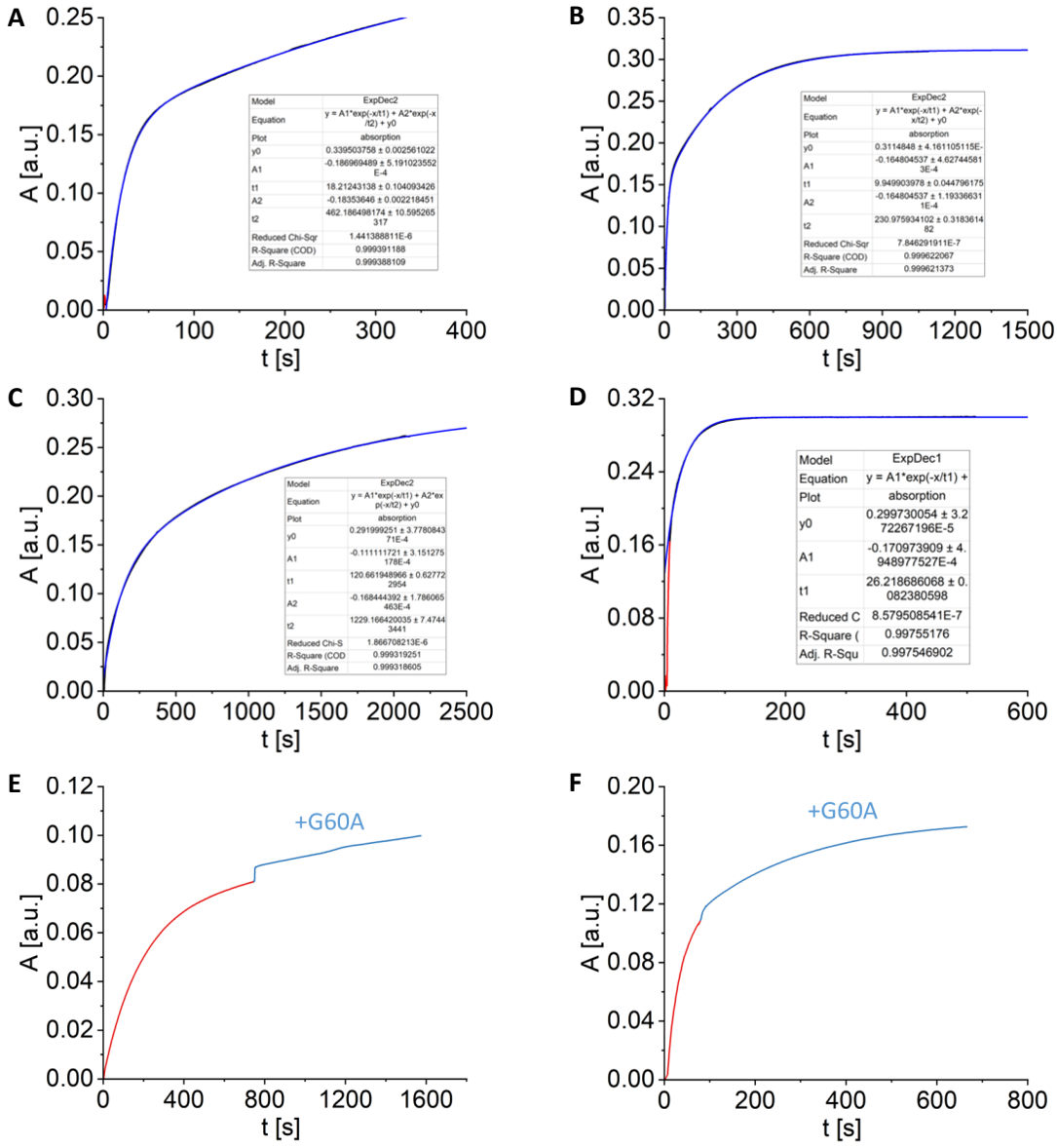

Figure S 34: Enzymatic hydrolysis of substrate III catalyzed by PTE_H257ONBY before and after illumination
A Time course for the hydrolysis of 10 µM III-S_P/R_P by 2 nM PTE_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic 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

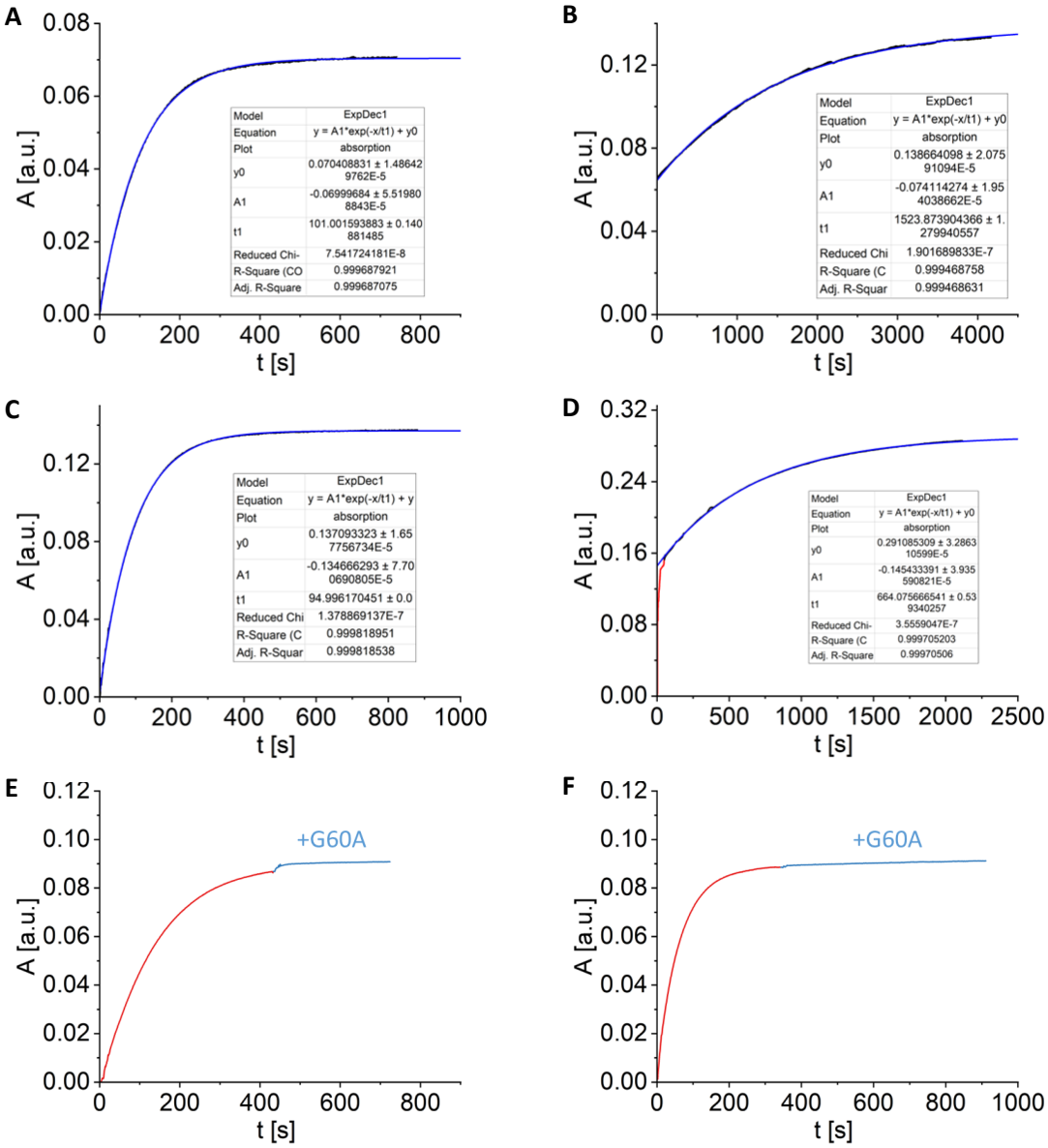

Figure S 35: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H257ONBY before and after illumination
A Time course for the hydrolysis of 10 µM IV-S_P/R_P by 10 nM PTE_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic 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

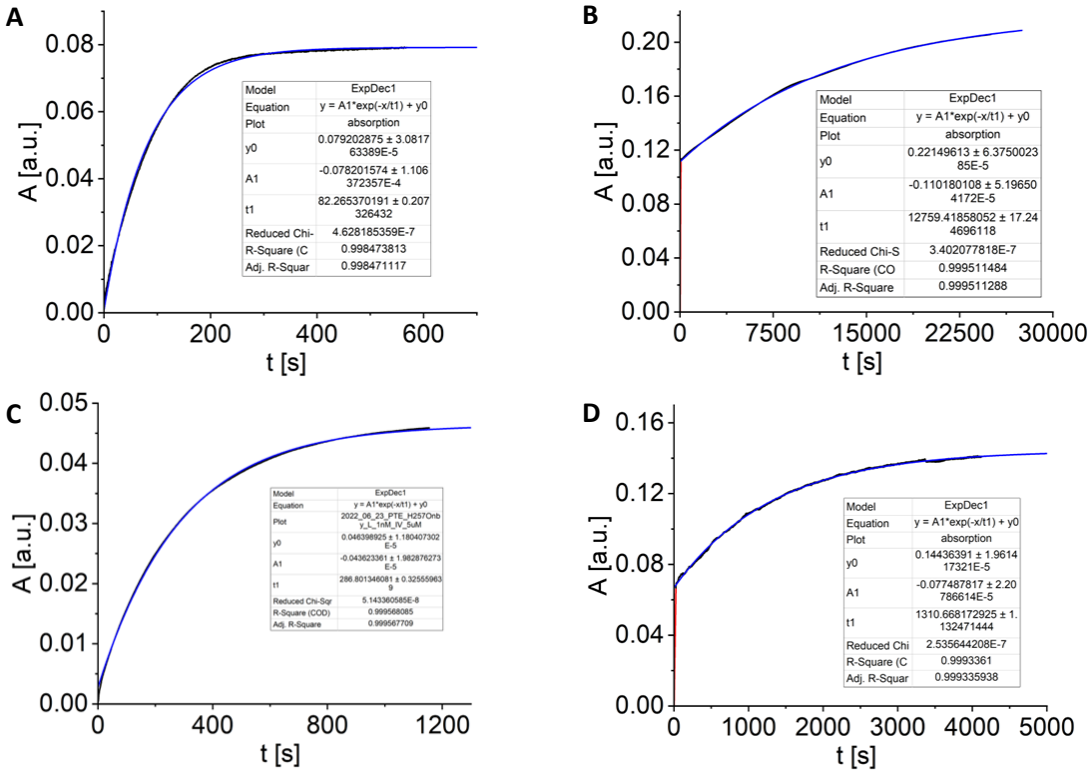

Figure S 36: Enzymatic hydrolysis of substrate V catalyzed by PTE_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 µM 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 µM V-S_P/R_P by 3.6 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 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 µM 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 µM V-S_P/R_P by 10 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 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

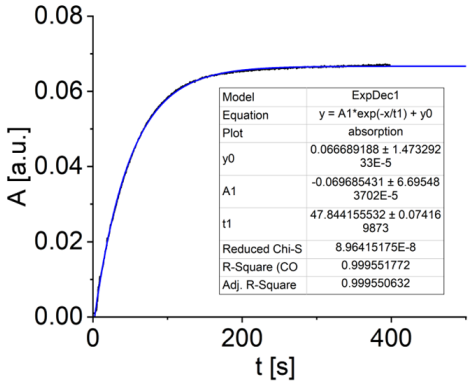

Figure S 37: Enzymatic hydrolysis of substrate I catalyzed by PTE_H257Y

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

Substrate II

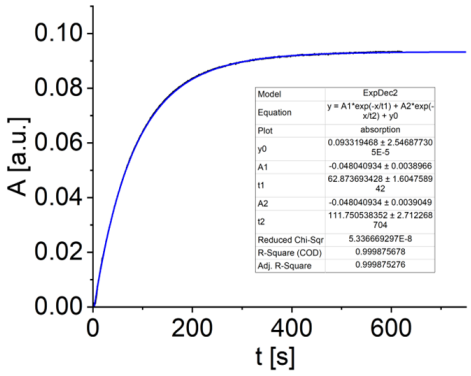

Figure S 38: Enzymatic hydrolysis of substrate II catalyzed by PTE_H257Y

A Time course for the hydrolysis of 5 µM 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

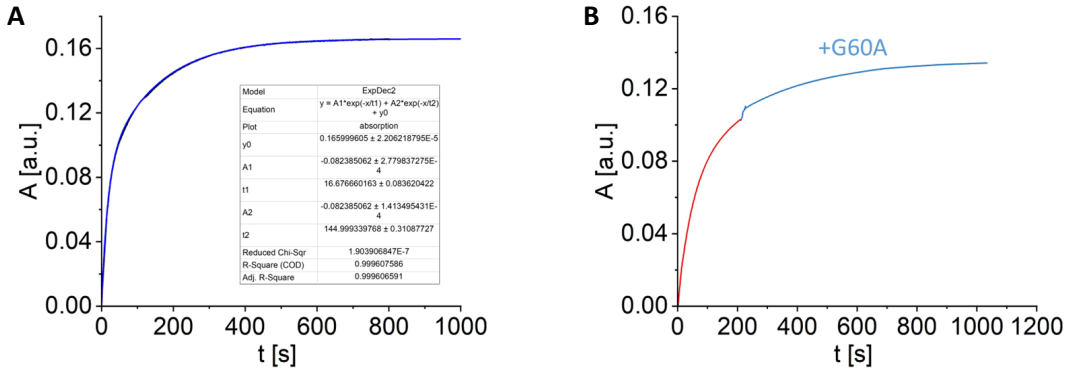

Figure S 39: Enzymatic hydrolysis of substrate III catalyzed by PTE_H257Y

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

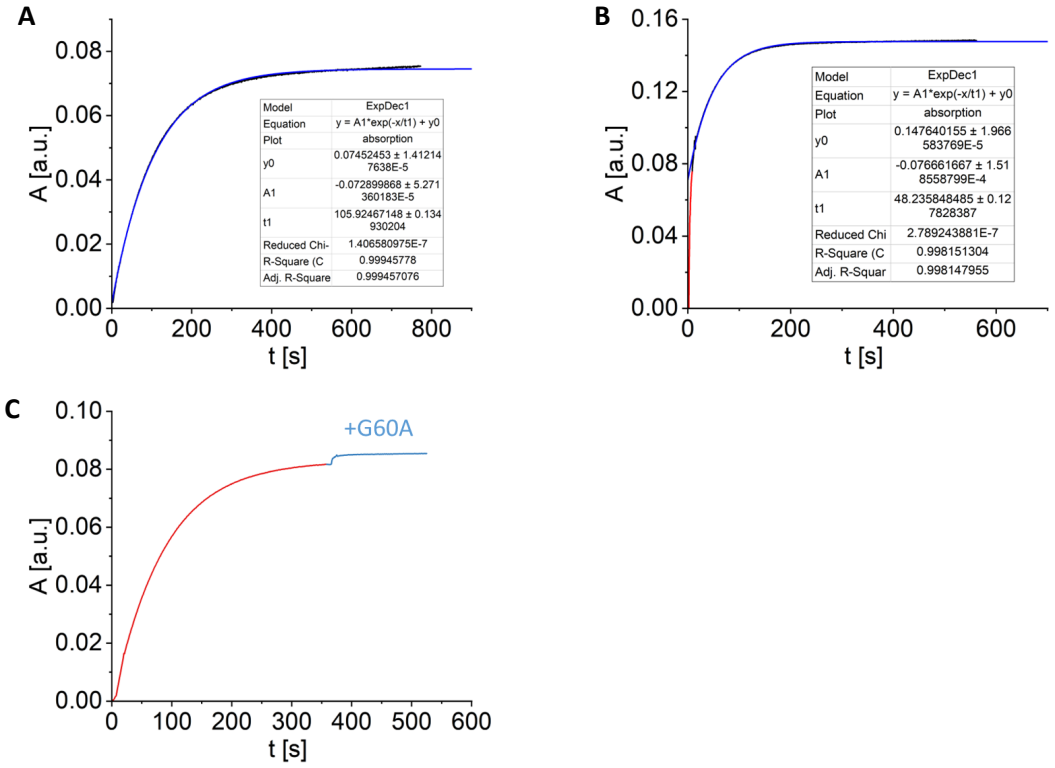

Figure S 40: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H257Y

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

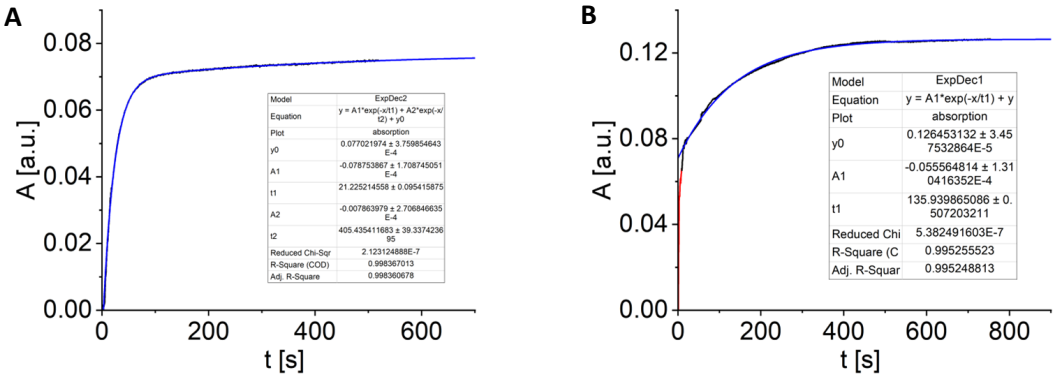

Figure S 41: Enzymatic hydrolysis of substrate V catalyzed by PTE_H257Y

A Time course for the hydrolysis of 10 µM 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 µM 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

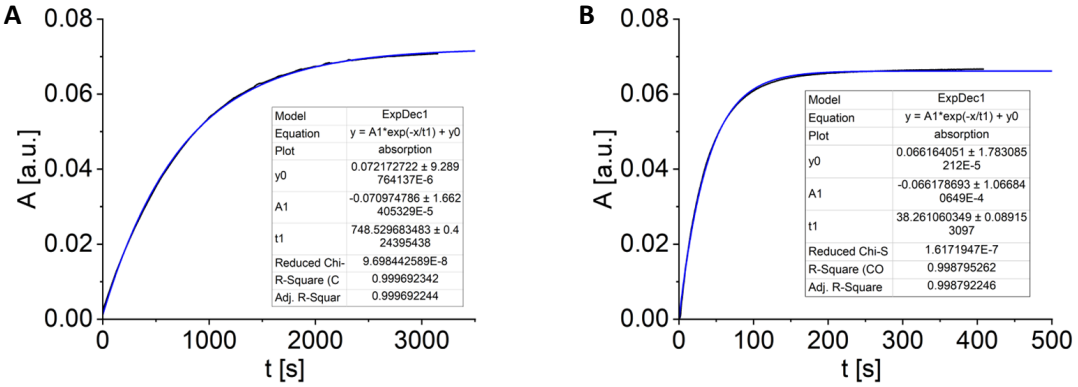

Figure S 42: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_H257ONBY before and after illumination

A Time course for the hydrolysis of 5 µM 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 µM 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

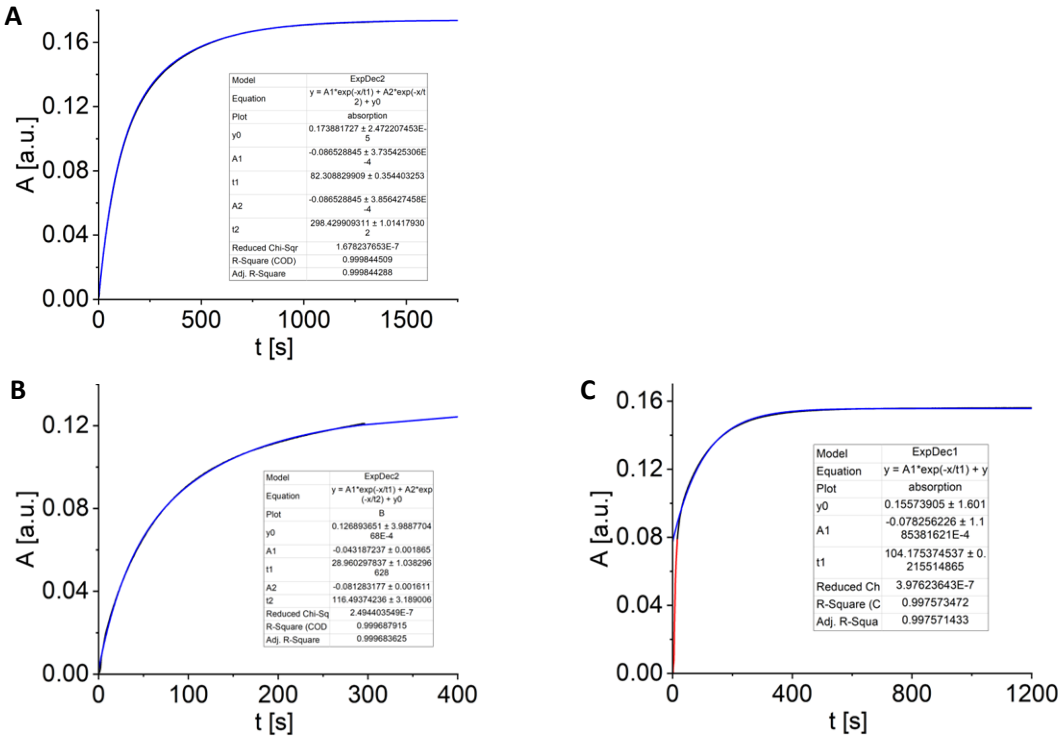

Figure S 43: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_H257ONBY before and after illumination
A Time course for the hydrolysis of 10 µM II-S_P/R_P by 10 nM PTE_I106A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of II-S_P/R_P. **B** Time course for the hydrolysis of 10 µM 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 µM 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

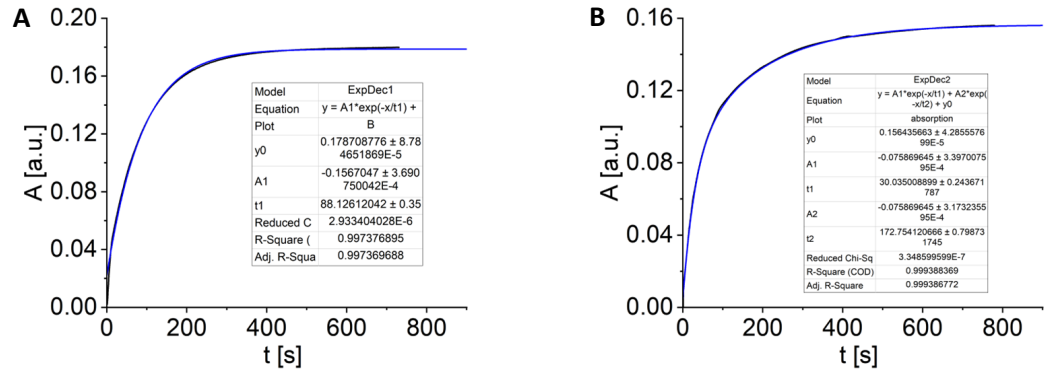

Figure S 44: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_H257ONBY before and after illumination
A Time course for the hydrolysis of 10 µM III-S_P/R_P by 5 nM PTE_I106A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257ONBY for the hydrolysis of III-S_P/R_P. **B** Time course for the hydrolysis of 10 µM 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

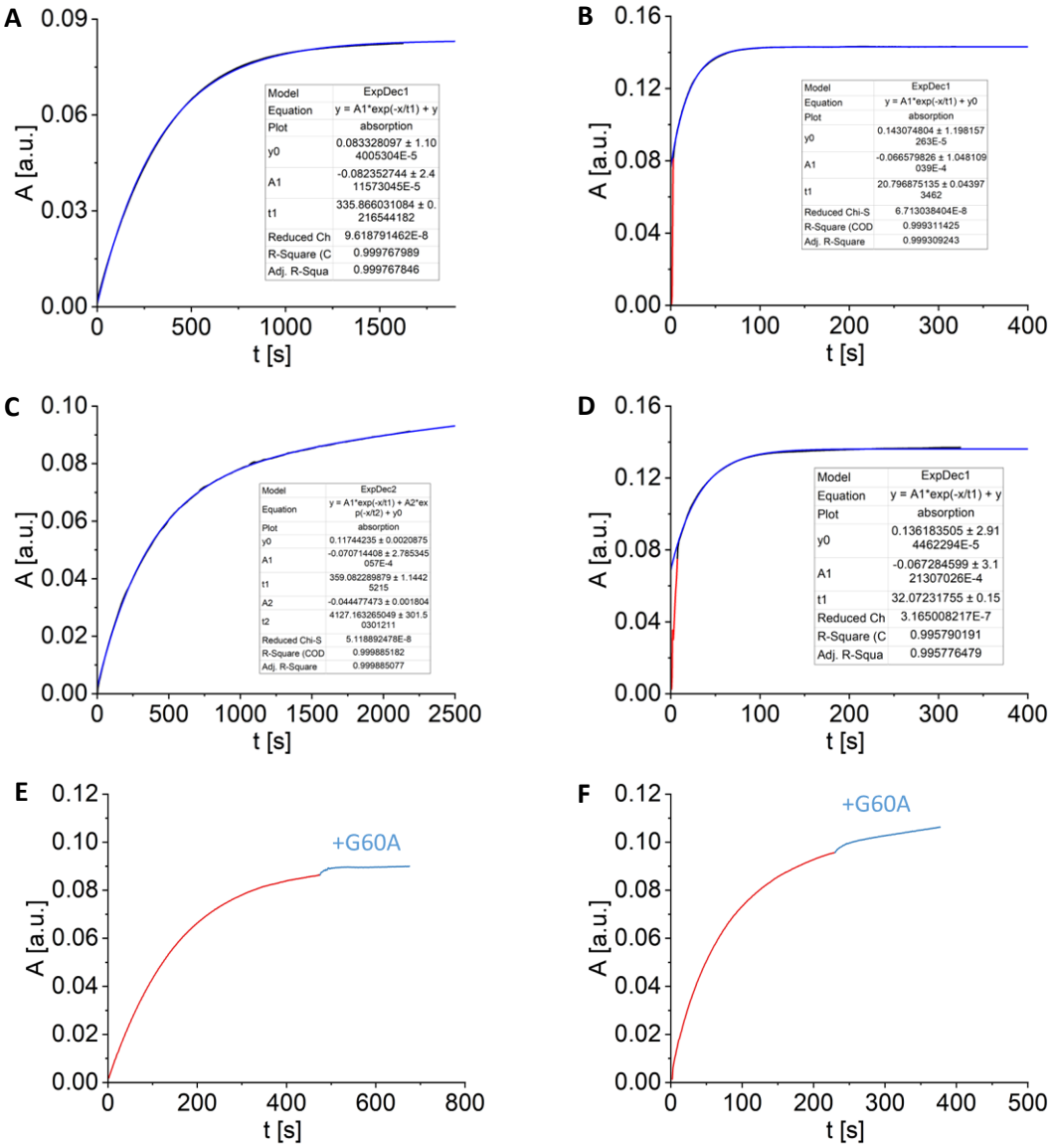

Figure S 45: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_H257ONBY before and after illumination
A Time course for the hydrolysis of 10 µM IV-S_P/R_P by 20 nM PTE_I106A_H257ONBY *as isolated* (black). The data was fitted to a single exponential function (blue) that was used to determine 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

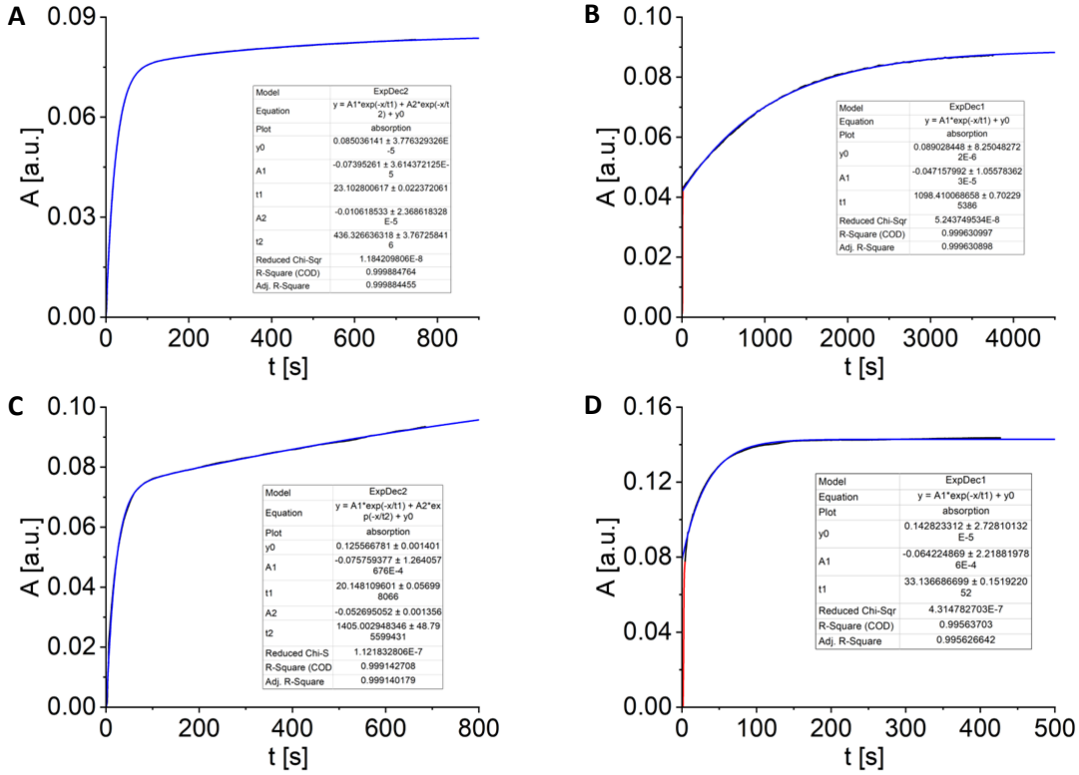

Figure S 46: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_H257ONBY before and after illumination
A Time course for the hydrolysis of 10 µM V-S_P/R_P by 40 nM PTE_I106A_H257ONBY *as isolated* (black). The data was fitted to a double exponential function (blue) that was used to determine 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

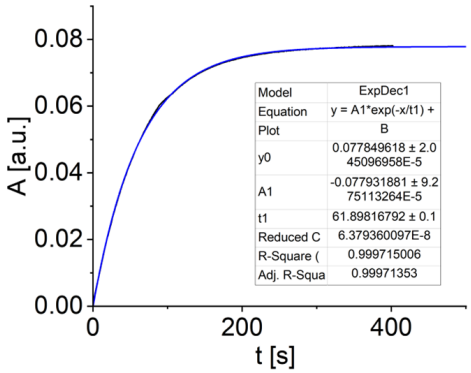

Figure S 47: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_H257Y
Time course for the hydrolysis of 5 µM substrate I by addition of 2 nM PTE_I106A_H257Y (black). The data was fitted to a single exponential function.

Substrate II

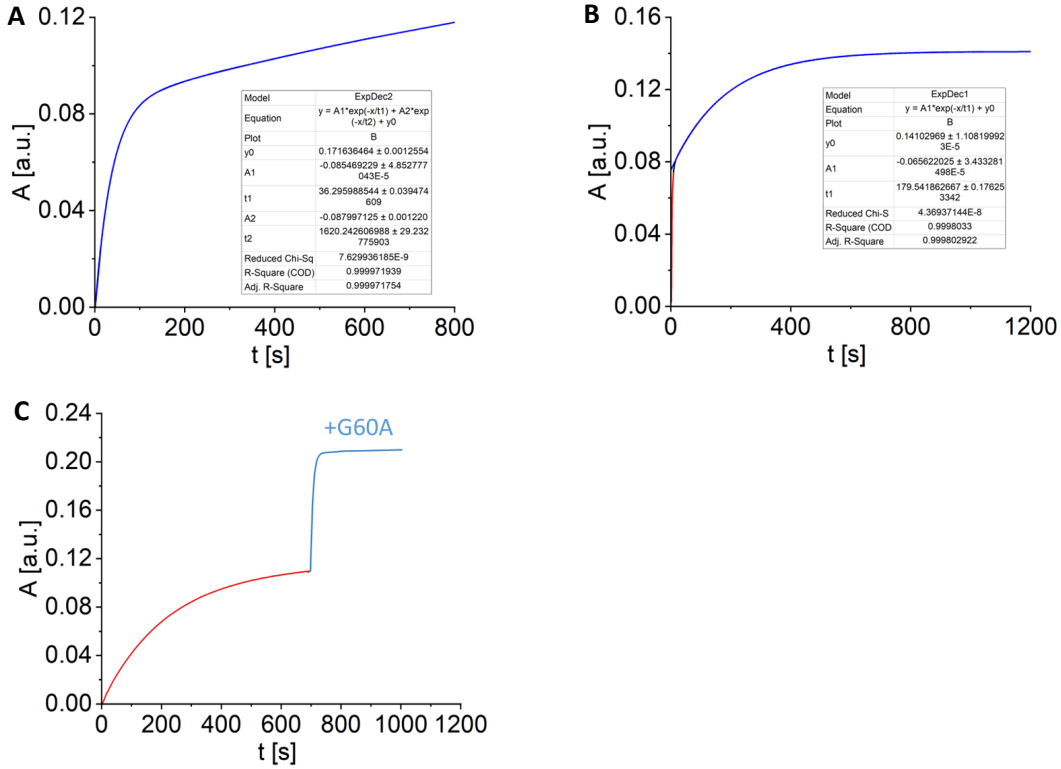

Figure S 48: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_H257Y
A Time course for the hydrolysis of 10 µM II- R_P/S_P by 0.5 nM PTE_I106A_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for 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 absorption upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed.

Substrate III

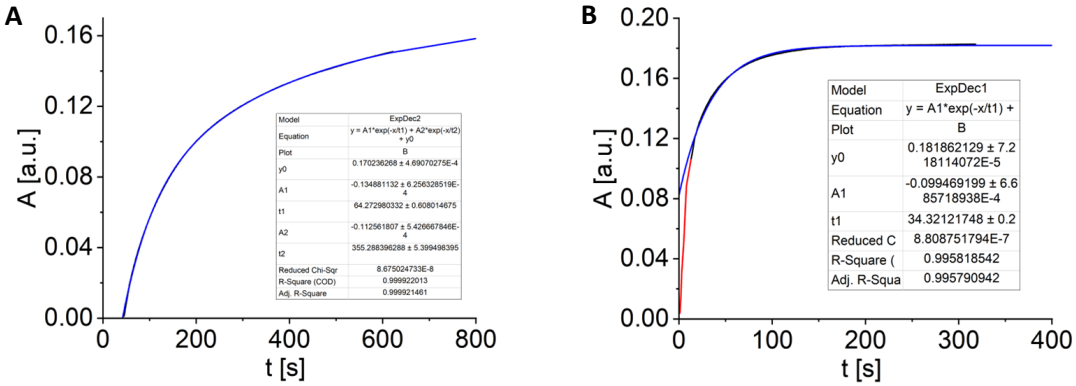

Figure S 49: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_H257Y

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

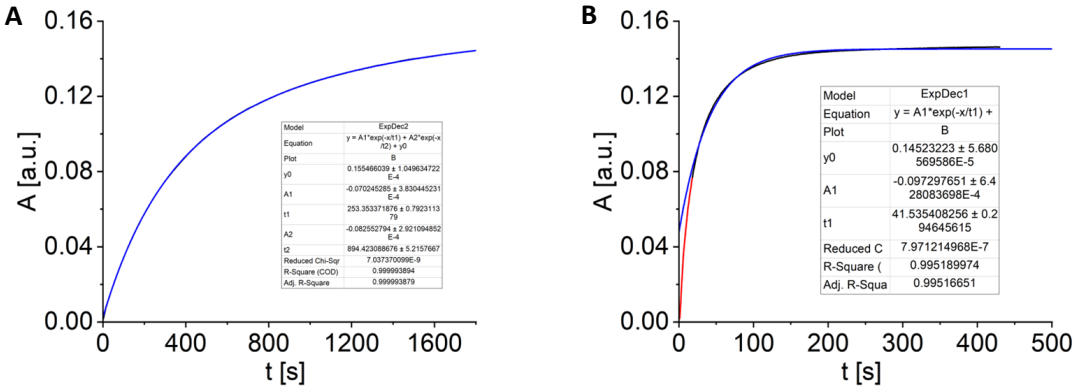

Figure S 50: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_H257Y

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

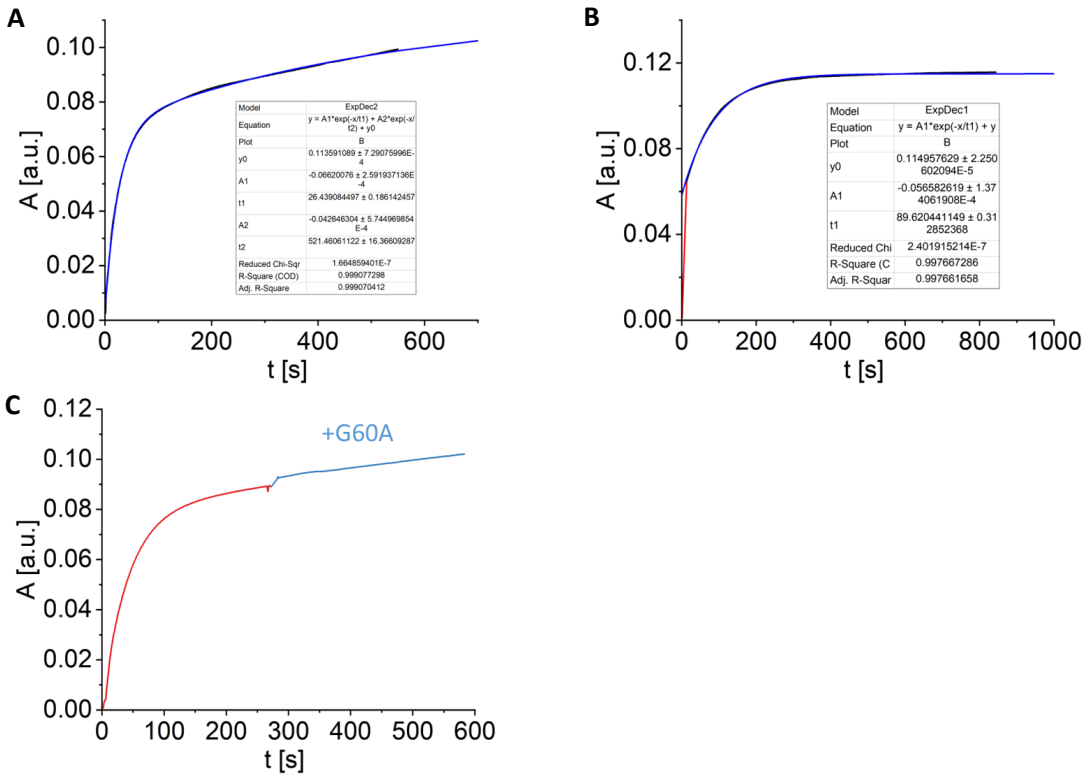

Figure S 51: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_H257Y

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

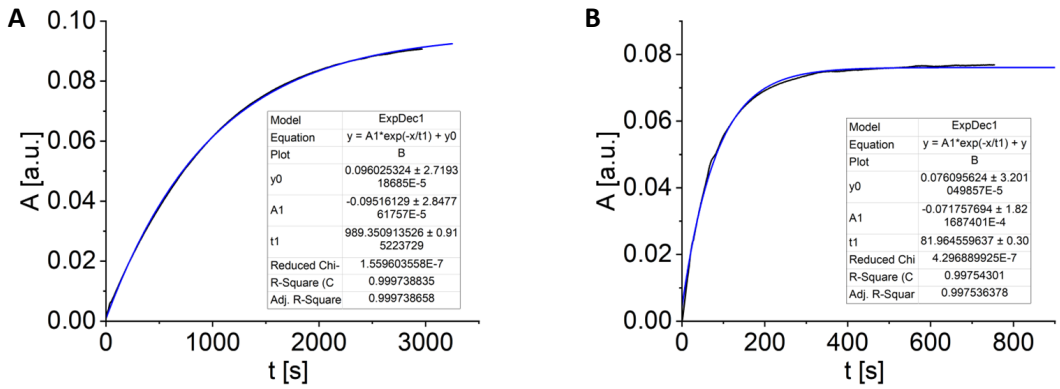

Figure S 52: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_F132A_H257ONBY before and after illumination

A Time course for the hydrolysis of 5 µM 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 µM 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

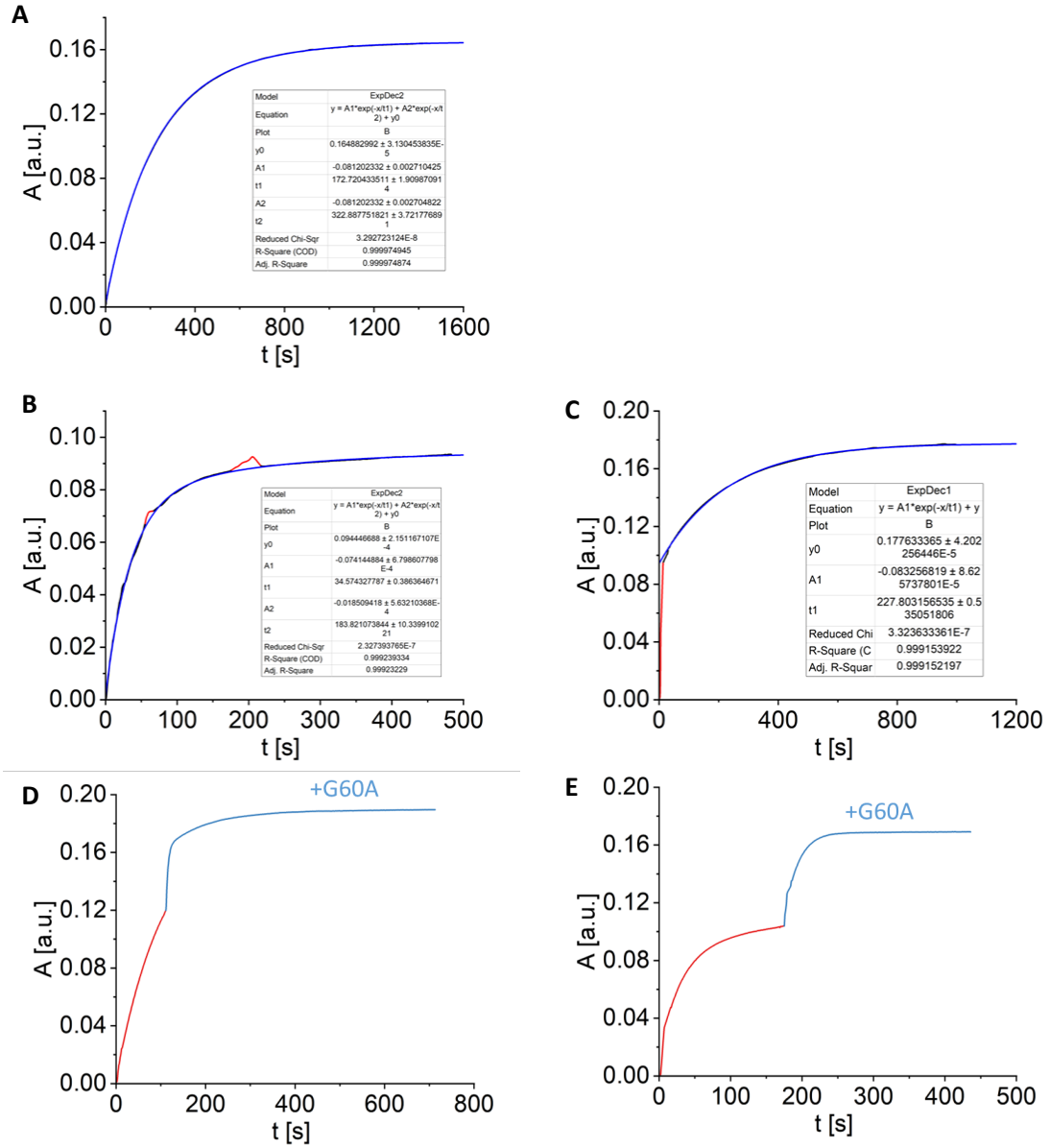

Figure S 53: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F132A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 µM 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 µM 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 µM 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 µM 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 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_H257ONBY after irradiation at 365 nm. Enzymatic hydrolysis of 10 µM 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 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

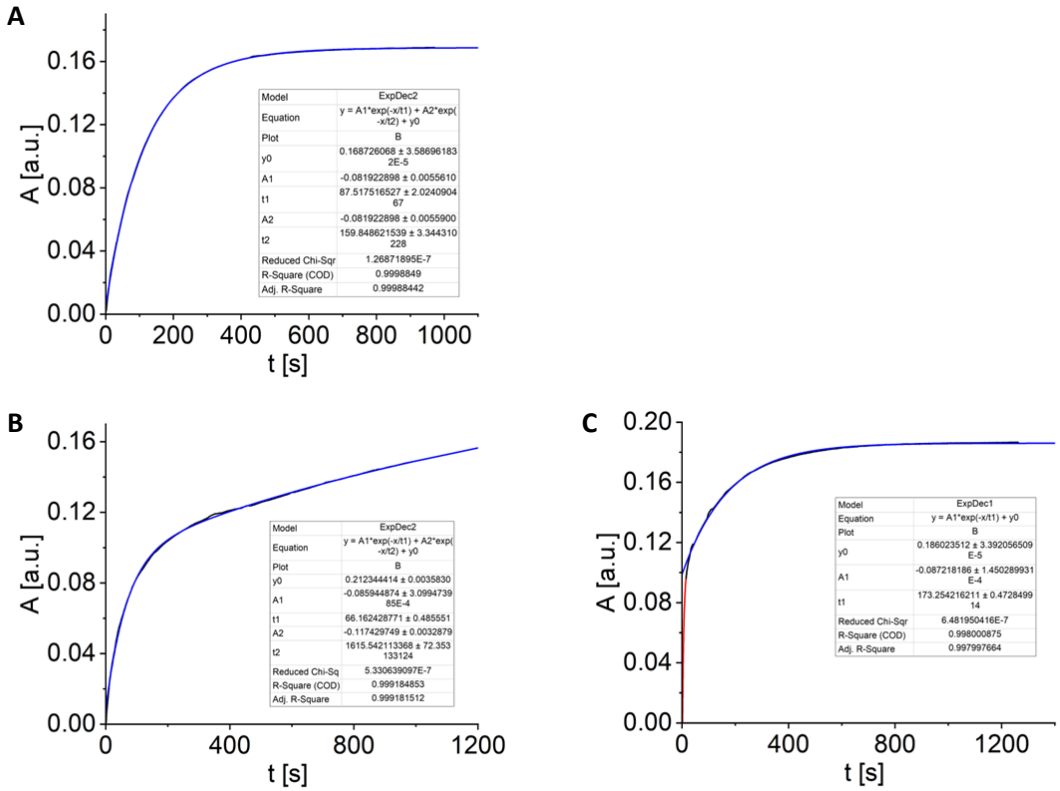

Figure S 54: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_H257ONBY before and after illumination

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

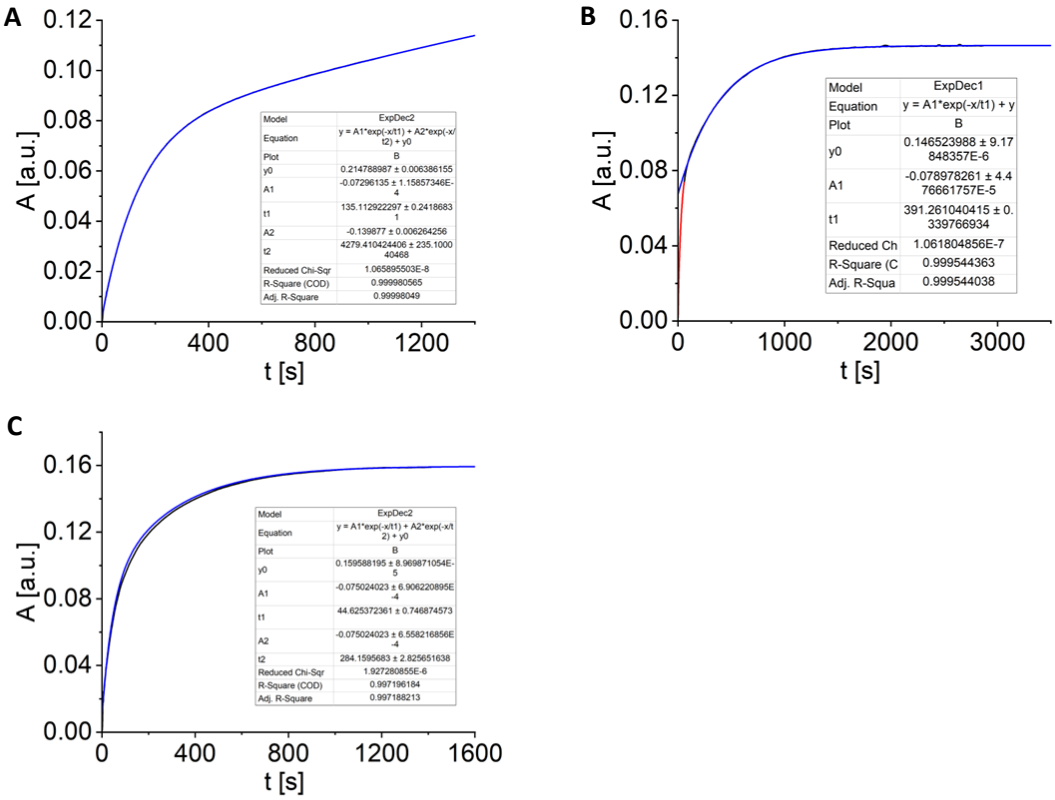

Figure S 55: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 µM 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 µM IV-S_P/R_P by 2 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 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_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

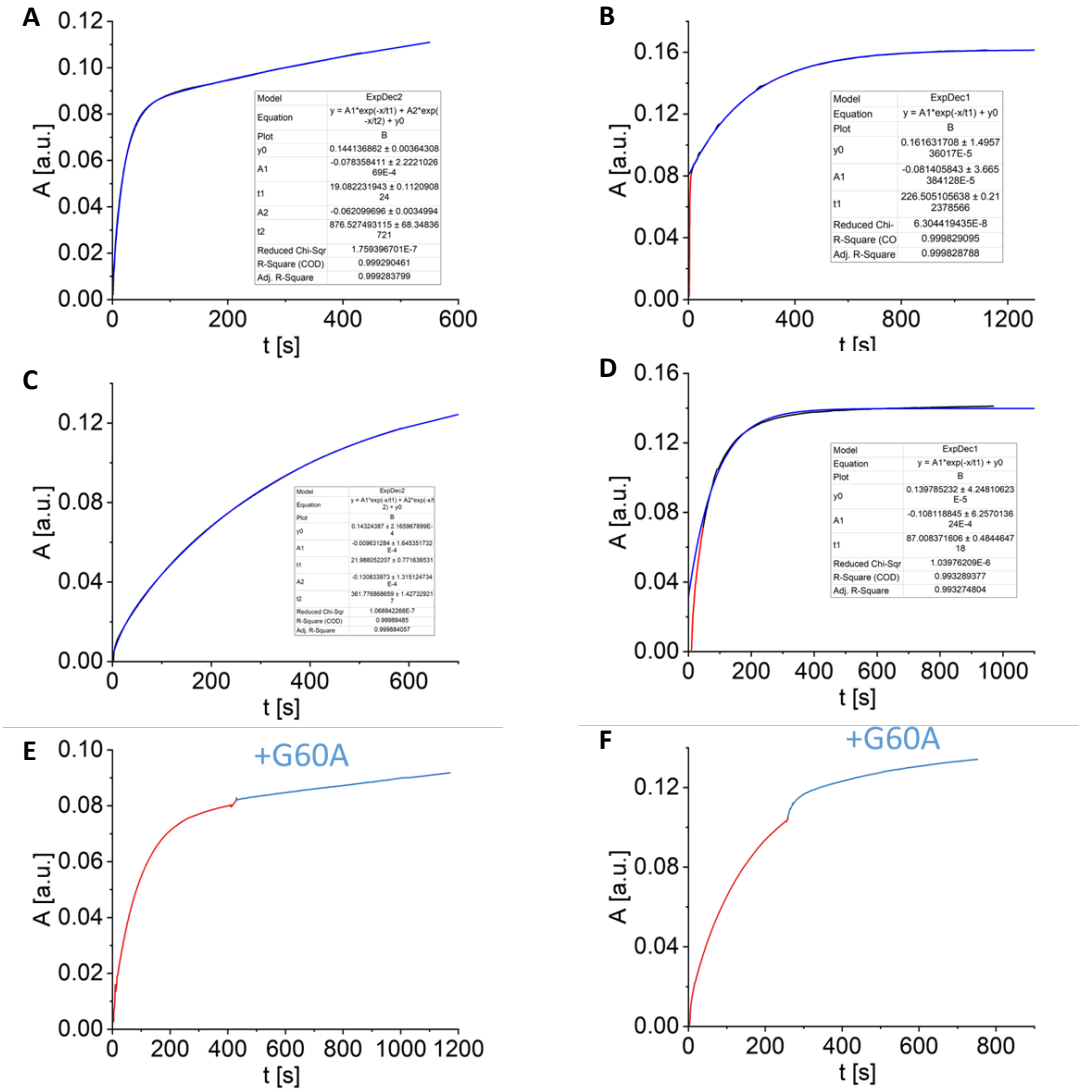

Figure S 56: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_H257ONBY before and after illumination

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

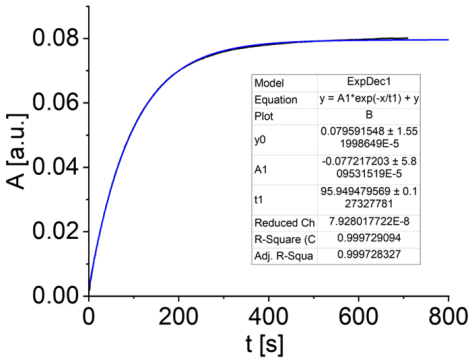

Figure S 57: Enzymatic hydrolysis of substrate I catalyzed by PTE_ I106A_F132A_H257Y
Time course for the hydrolysis of 5 µM substrate I by addition of 1 nM PTE_I106A_F132Y_H257Y (black). The data was fitted to a single exponential function.

Substrate II

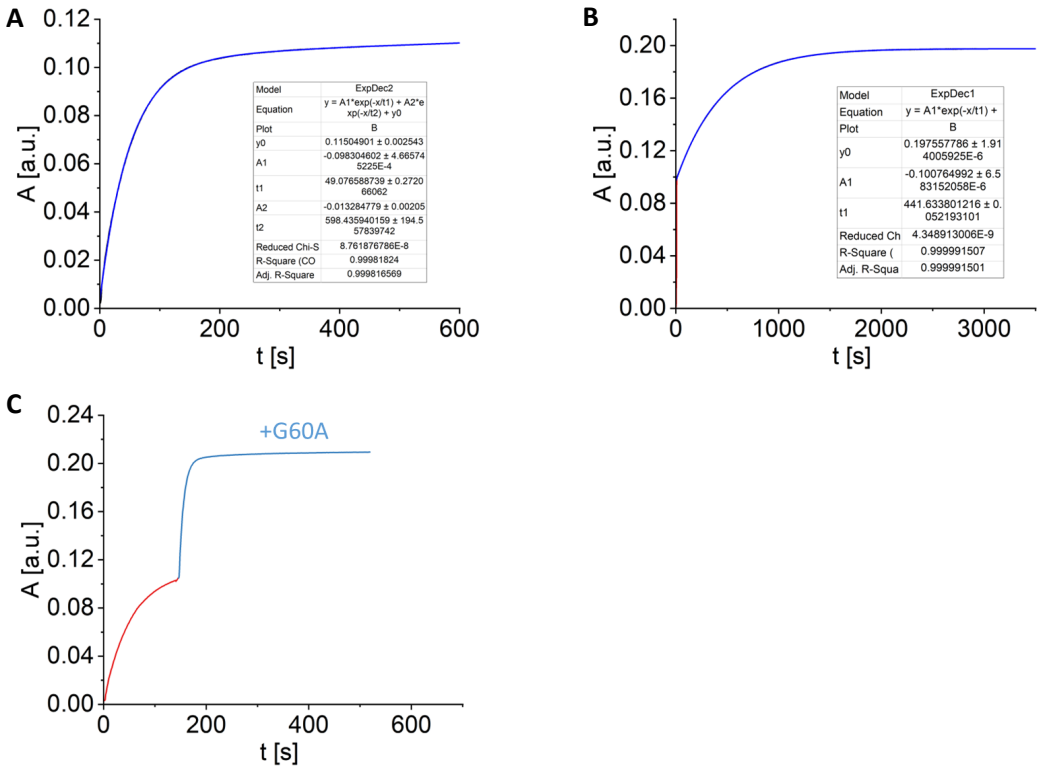

Figure S 58: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F123A_H257Y
A Time course for the hydrolysis of 10 µM II- R_P/S_P by 0.5 nM PTE_I106A_F132A_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H257Y 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 absorption upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed.

Substrate III

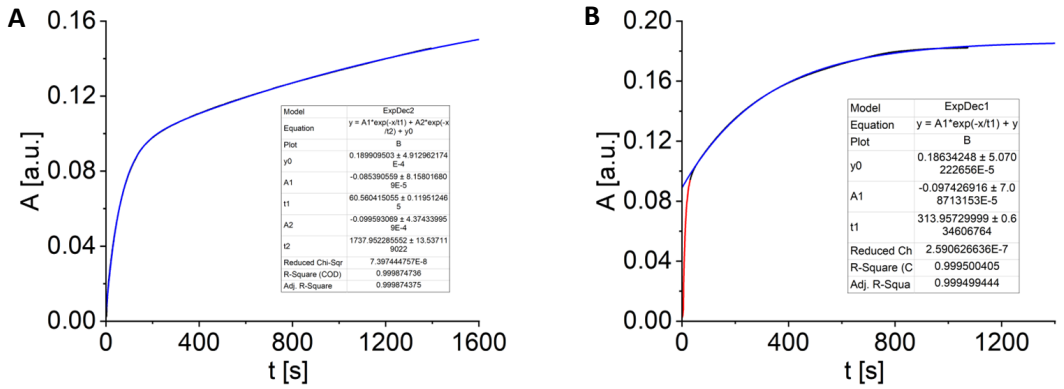

Figure S 59: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_H257Y

A Time course for the hydrolysis of 10 µM 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 µM 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

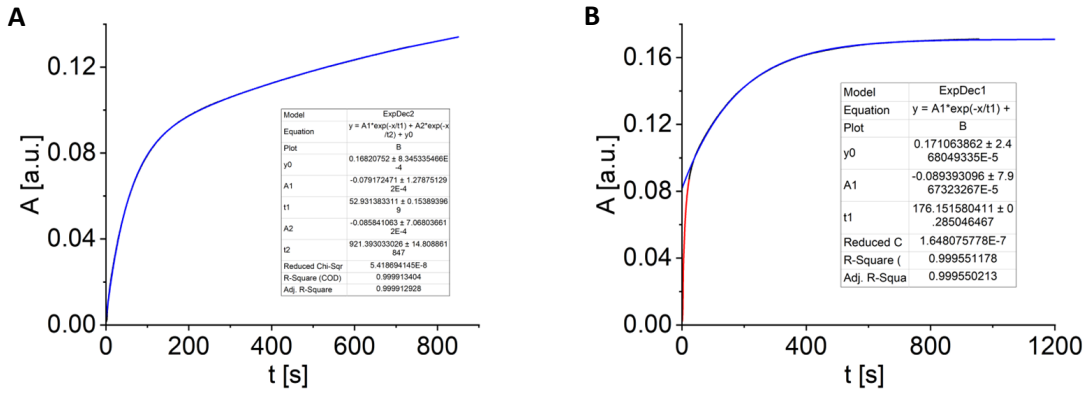

Figure S 60: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_H257Y

A Time course for the hydrolysis of 10 µM 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 µM 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

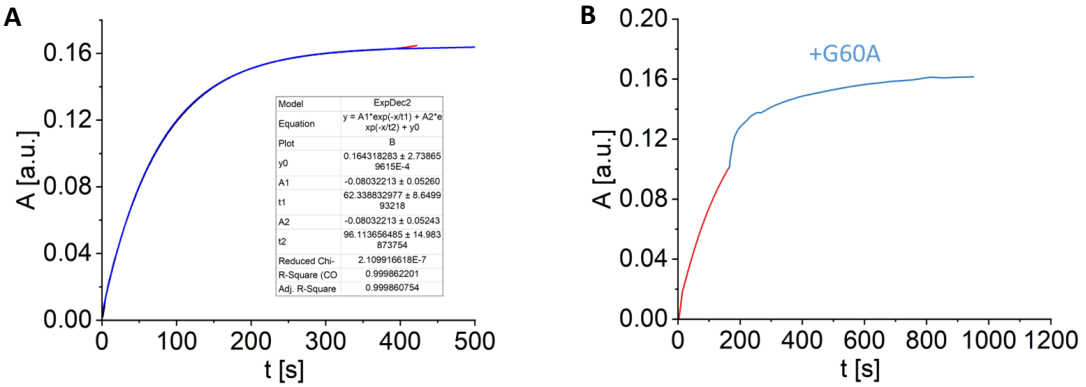

Figure S 61: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_H257Y

A Time course for the hydrolysis of 10 µM 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 µM 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

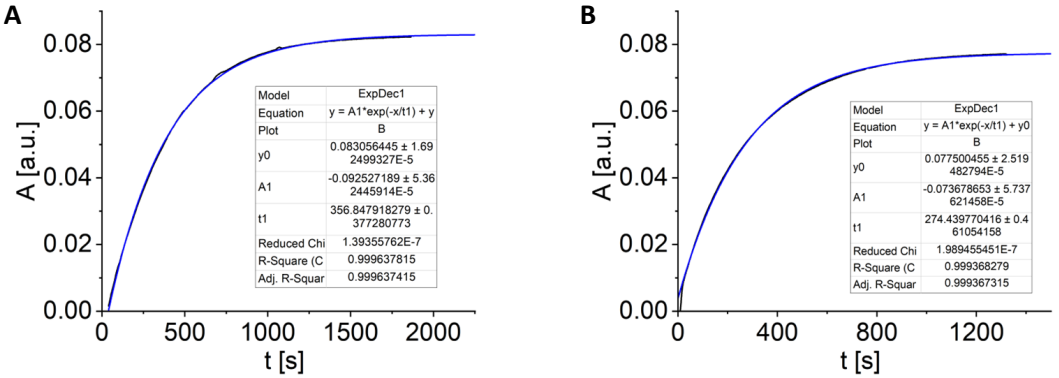

Figure S 62: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 5 µM 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 µM 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

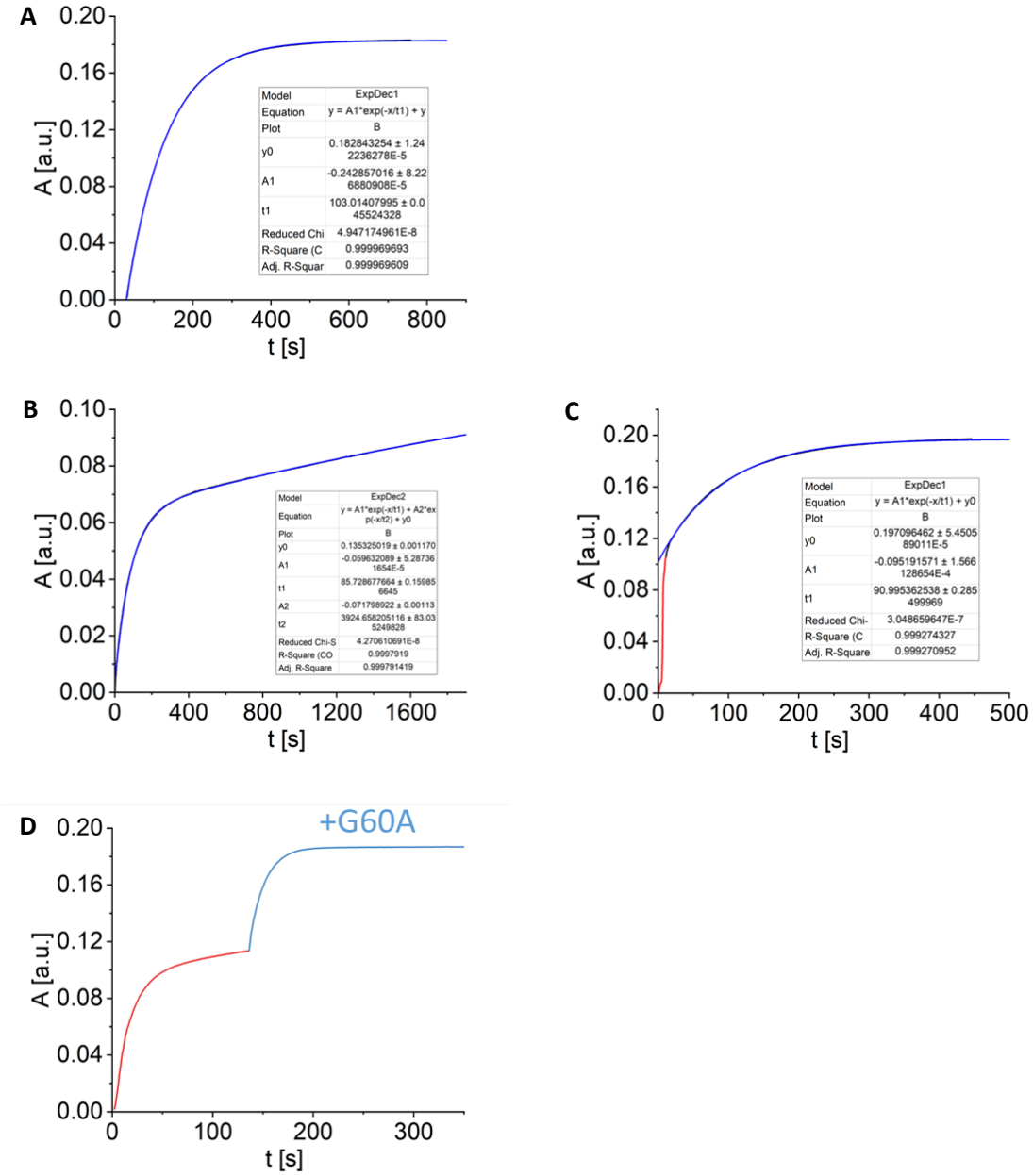

Figure S 63: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_S308A_H257ONBY before and after illumination

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

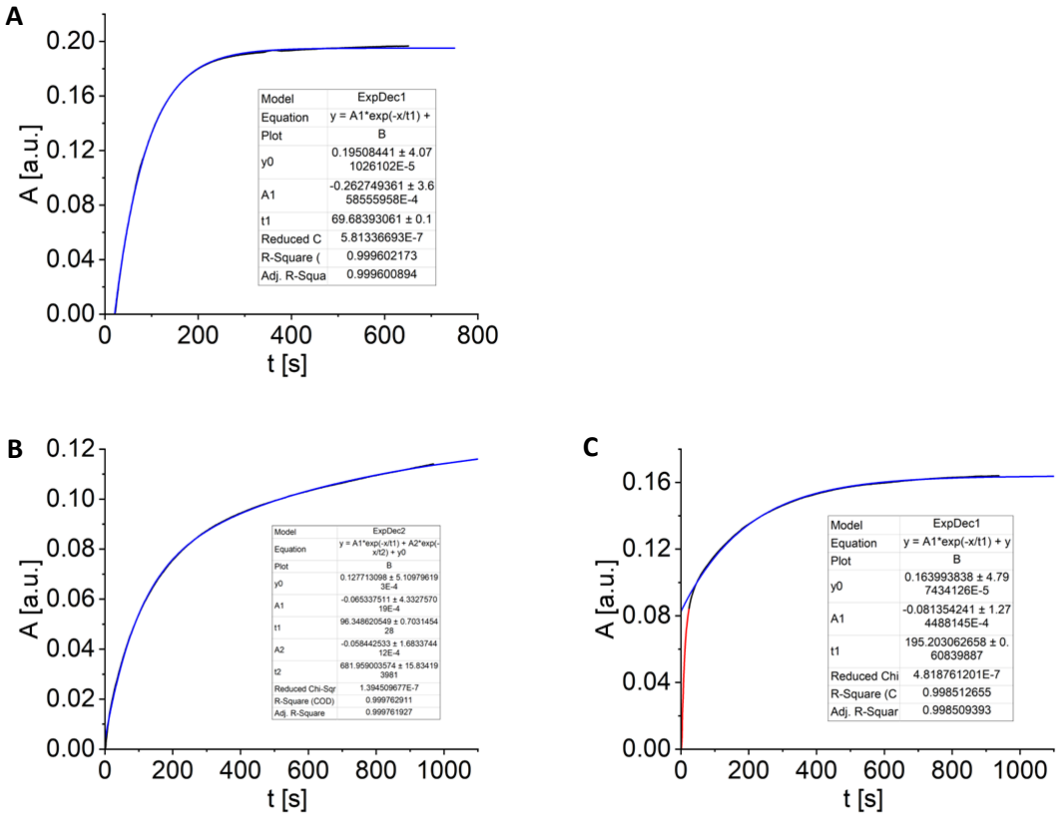

Figure S 64: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 µM 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 µM 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 µM 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

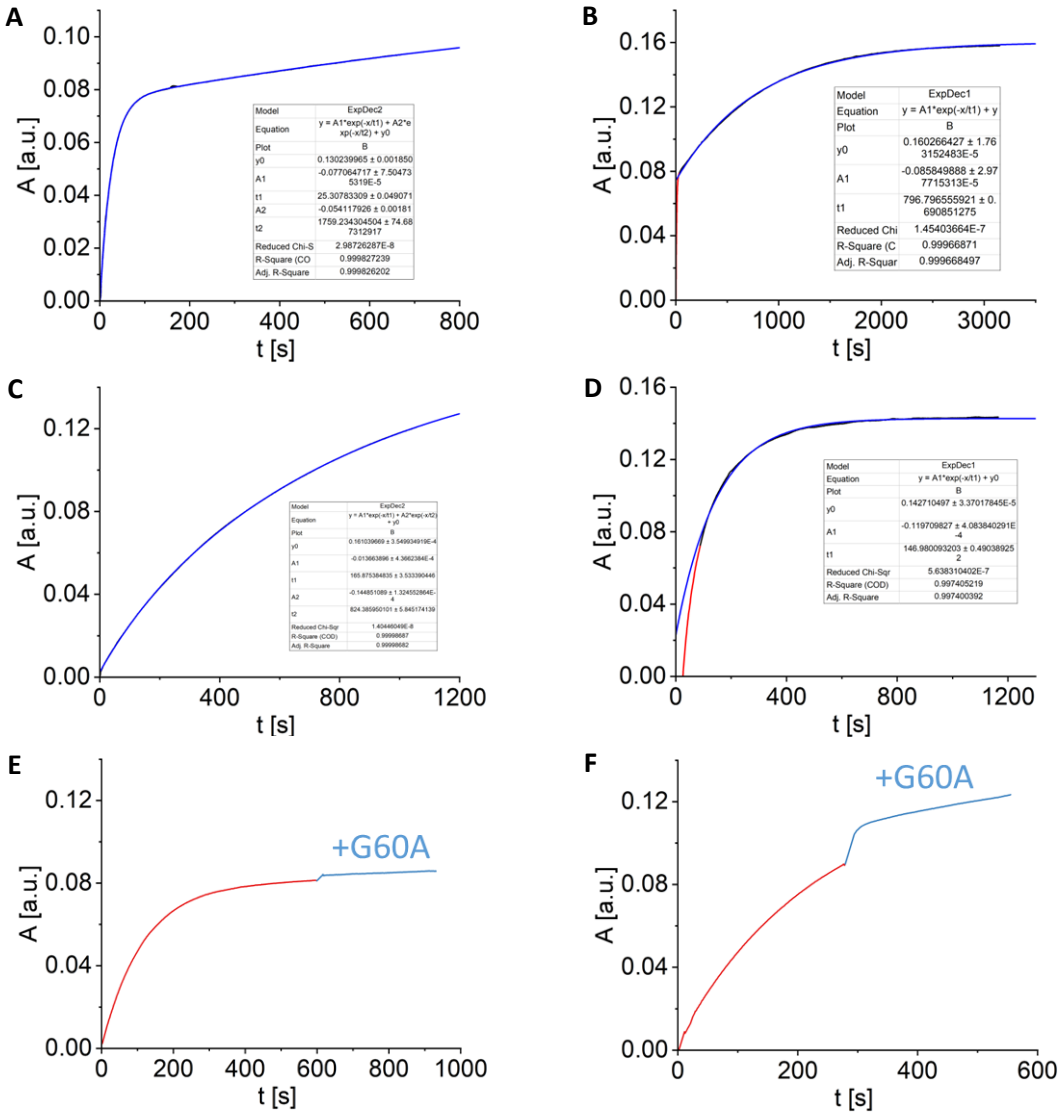

Figure S 65: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_S308_H257ONBY before and after illumination

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_308A_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 absorption is ovserved 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

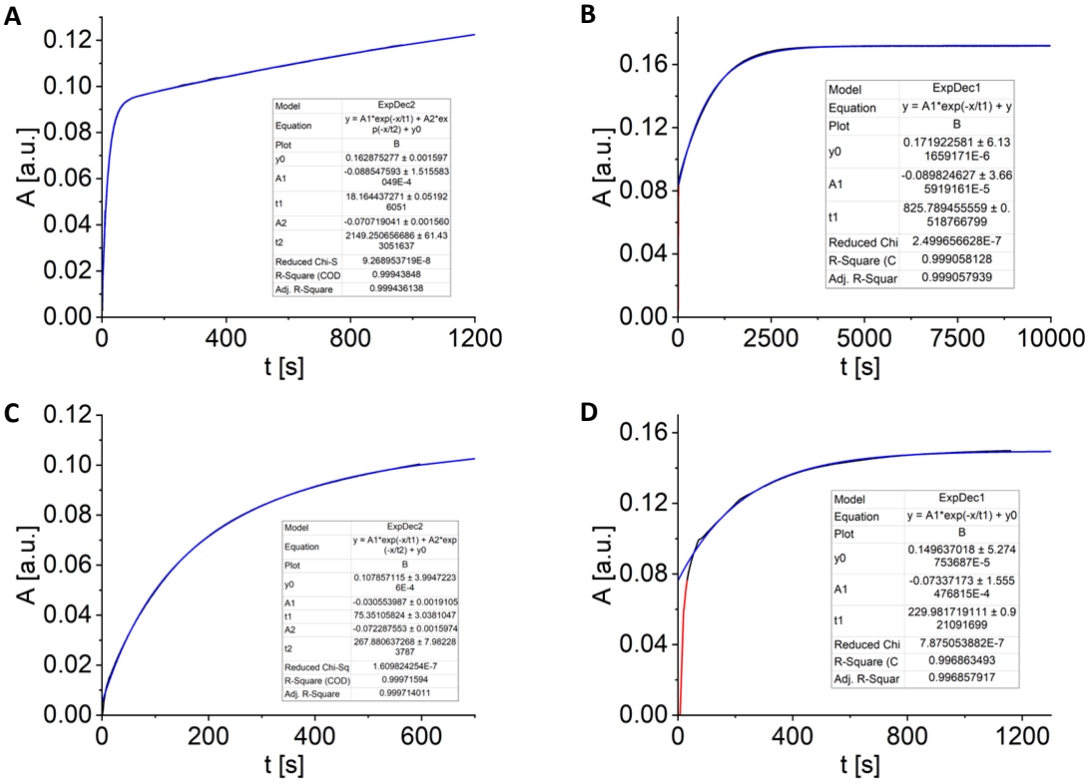

Figure S 66: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_S308A_H257ONBY before and after illumination

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

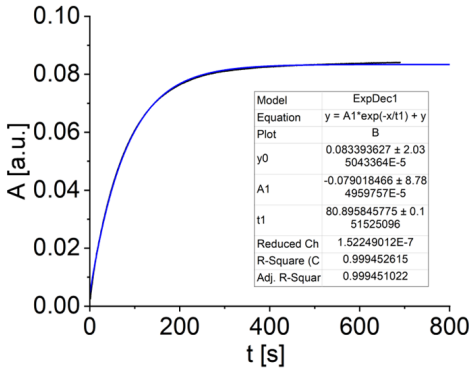

Figure S 67: Enzymatic hydrolysis of substrate I catalyzed by PTE_ I106A_H257Y_S308A
Time course for the hydrolysis of 5 µM substrate I by addition of 2 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a single exponential function.

Substrate II

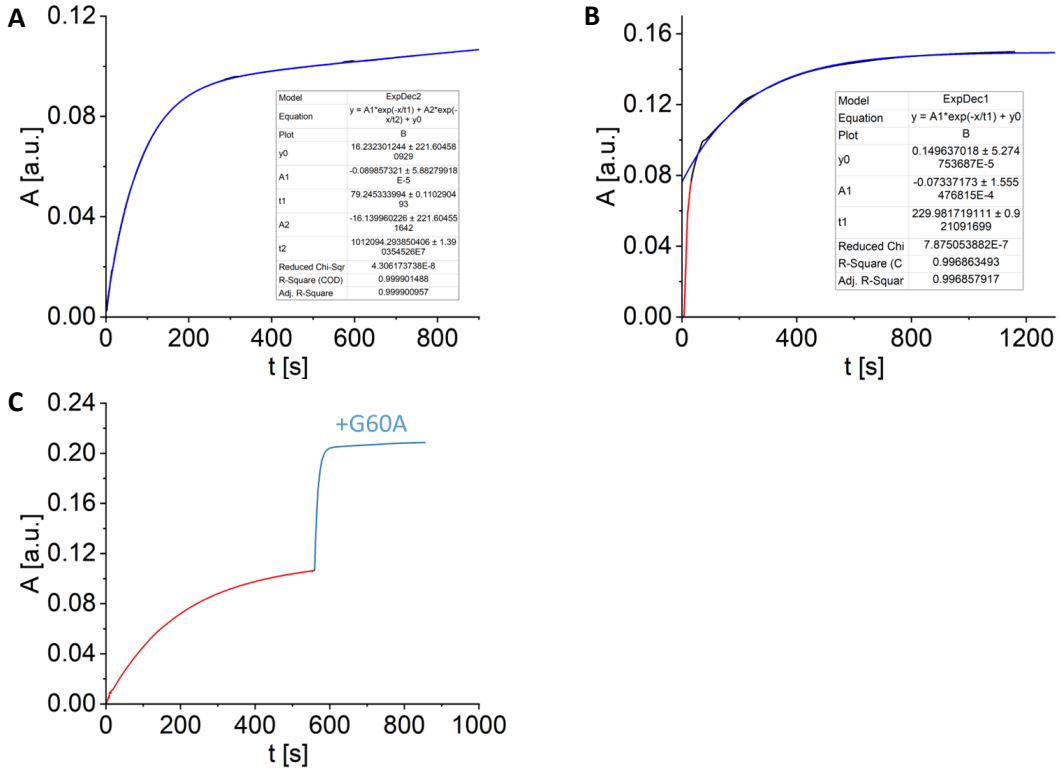

Figure S 68: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_H257Y_S308A
A Time course for the hydrolysis of 10 µM II- R_P/S_P by 0.5 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y for the hydrolysis of II-R_P. **B** Time course for the hydrolysis of 10 µM 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 µM 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 absorption upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed.

Substrate III

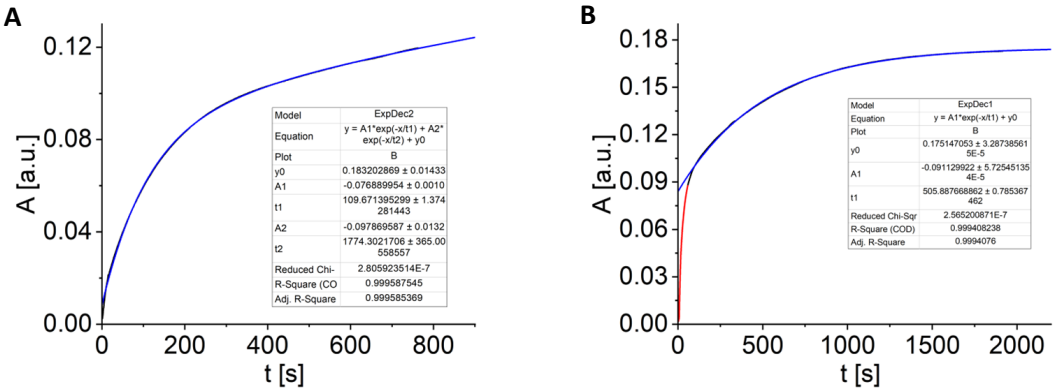

Figure S 69: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_H257Y_S308A
A Time course for the hydrolysis of 10 µM III-S_P/R_P by 0.5 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y_S308A 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

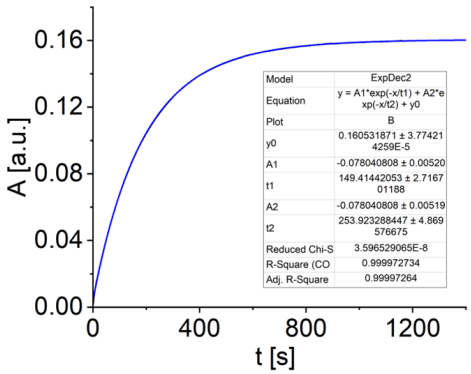

Figure S 70: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_H257Y_S308A
A Time course for the hydrolysis of 10 µM IV-S_P/R_P by 100 nM PTE_I106A_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_H257Y_S308A for the hydrolysis of IV-S_P/R_P.

Substrate V

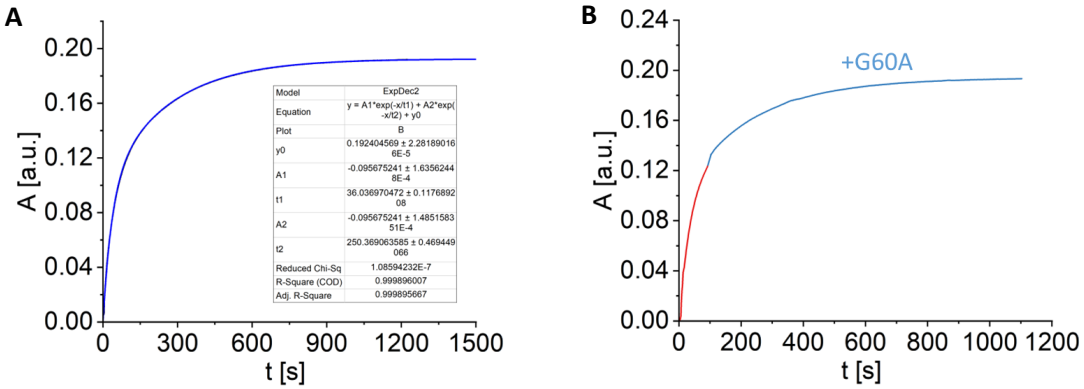

Figure S 71: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_H257Y_S308A

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

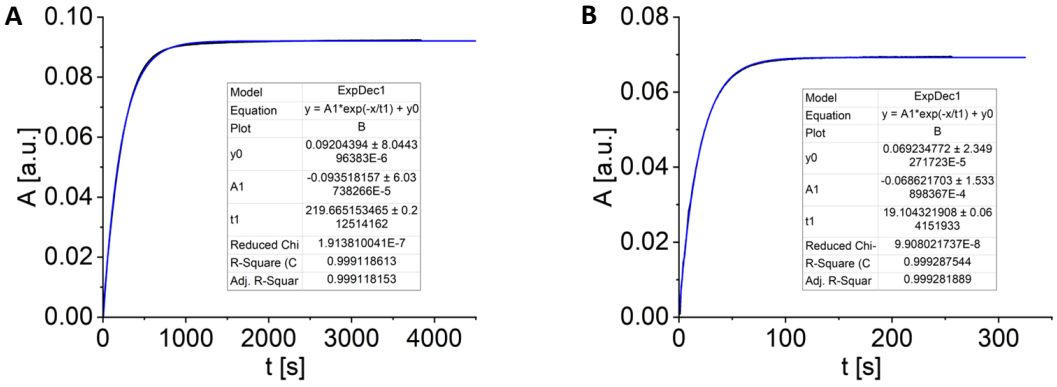

Figure S 72: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_F132A_H254G_H257ONBY before and after illumination

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 fucntion (blue).

Substrate II

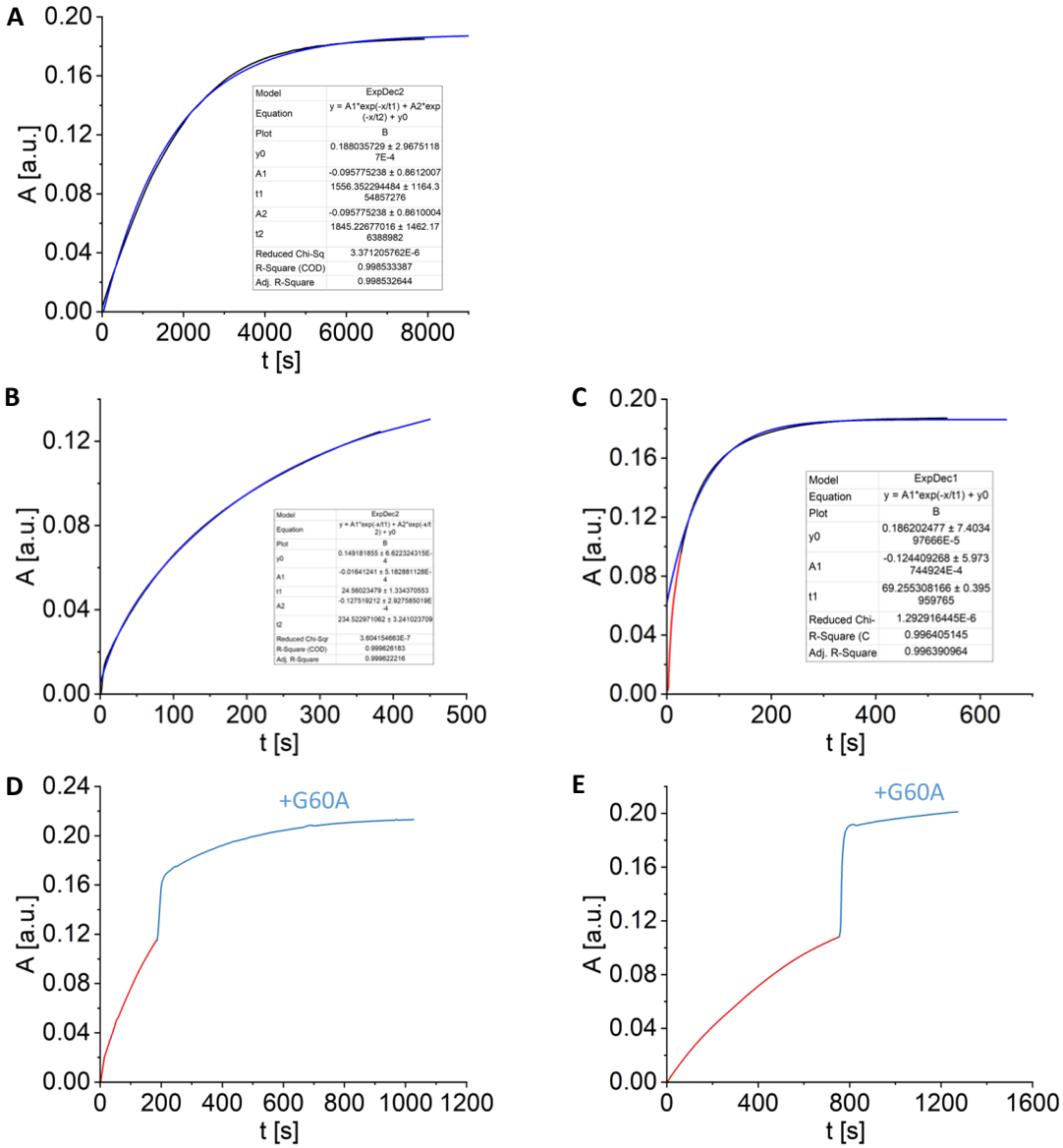

Figure S 73: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F132A_H254G_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 µM 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 µM 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 µM 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 µM 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 µM 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

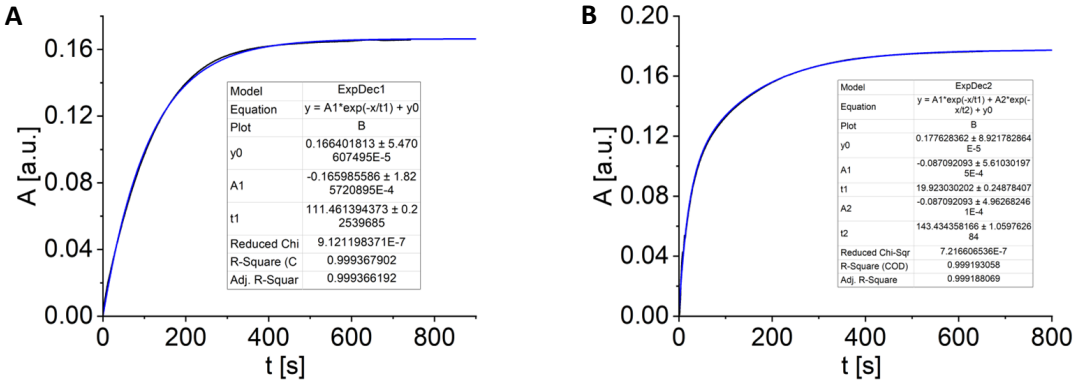

Figure S 74: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_H254G_H257ONBY before and after illumination

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

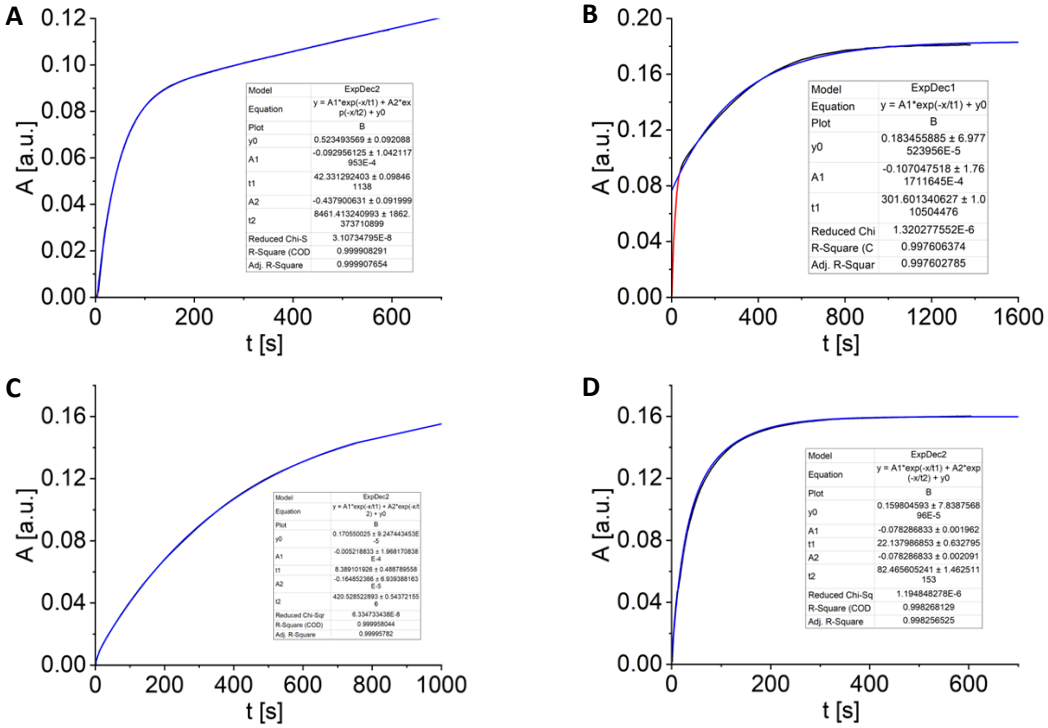

Figure S 75: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_H254G_H257ONBY before and after illumination

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

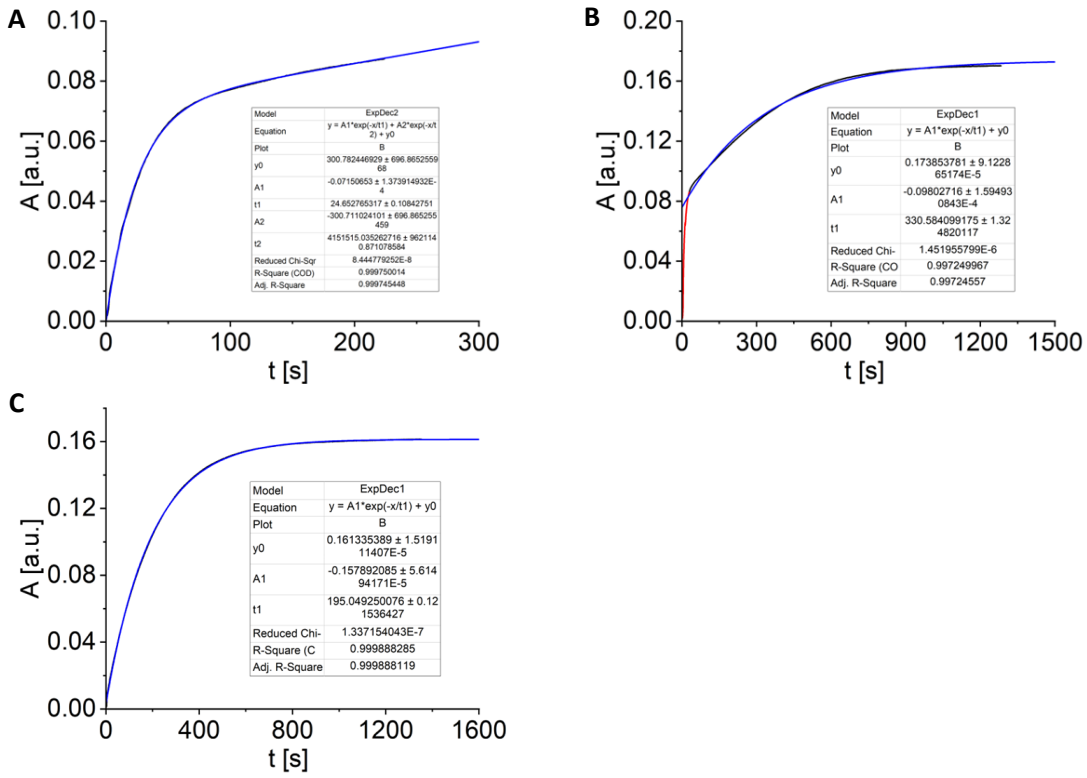

Figure S 76: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_H254G_H257ONBY before and after illumination

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

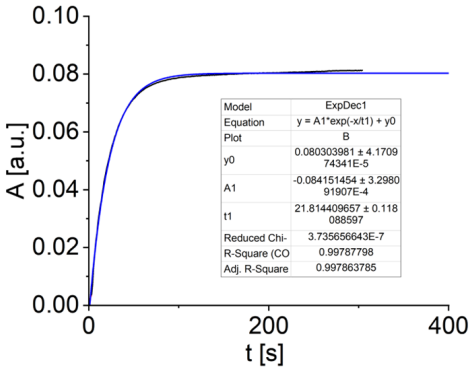

Figure S 77: Enzymatic hydrolysis of substrate I catalyzed by PTE_ I106A_F132A_H254G_H257Y
Time course for the hydrolysis of 5 µM substrate I by addition of 5 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a single exponential function.

Substrate II

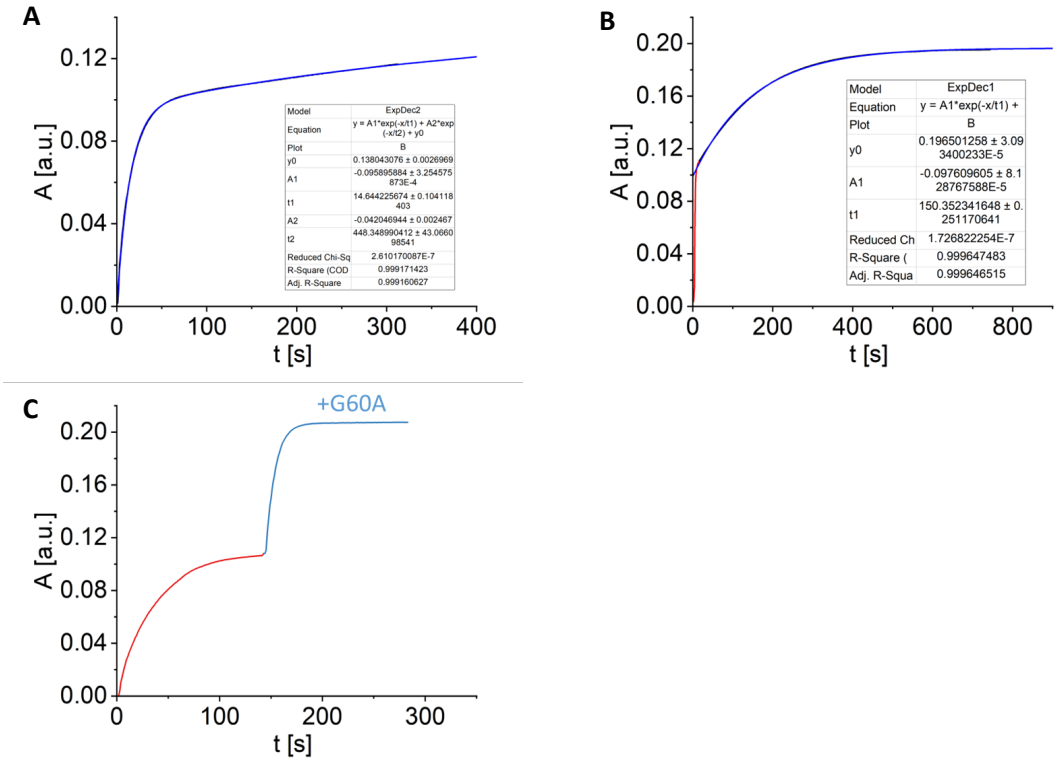

Figure S 78: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F123A_H254G_H257Y
A Time course for the hydrolysis of 10 µM II- R_P/S_P by 10 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of II-R_P. **B** Time course for the hydrolysis of 10 µM 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 (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 µM 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 absorption upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed.

Substrate III

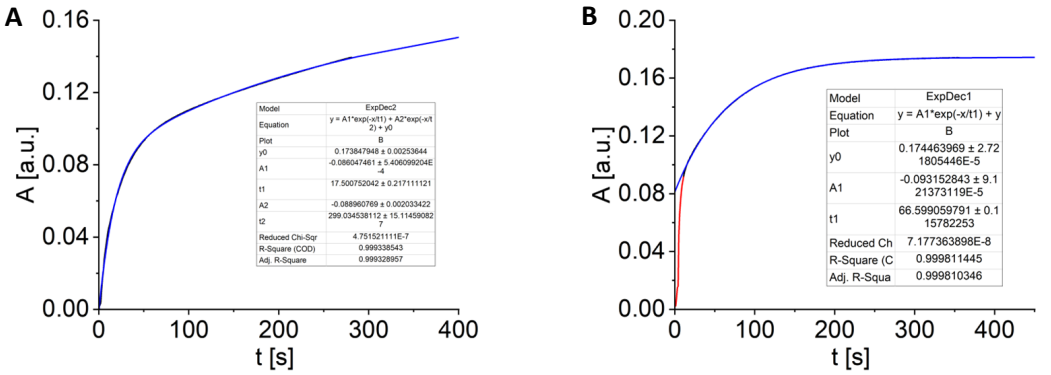

Figure S 79: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_H254G_H257Y
A Time course for the hydrolysis of 10 µM III-S_P/R_P by 5 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of III-R_P. **B** Time course for the hydrolysis of 10 µM 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

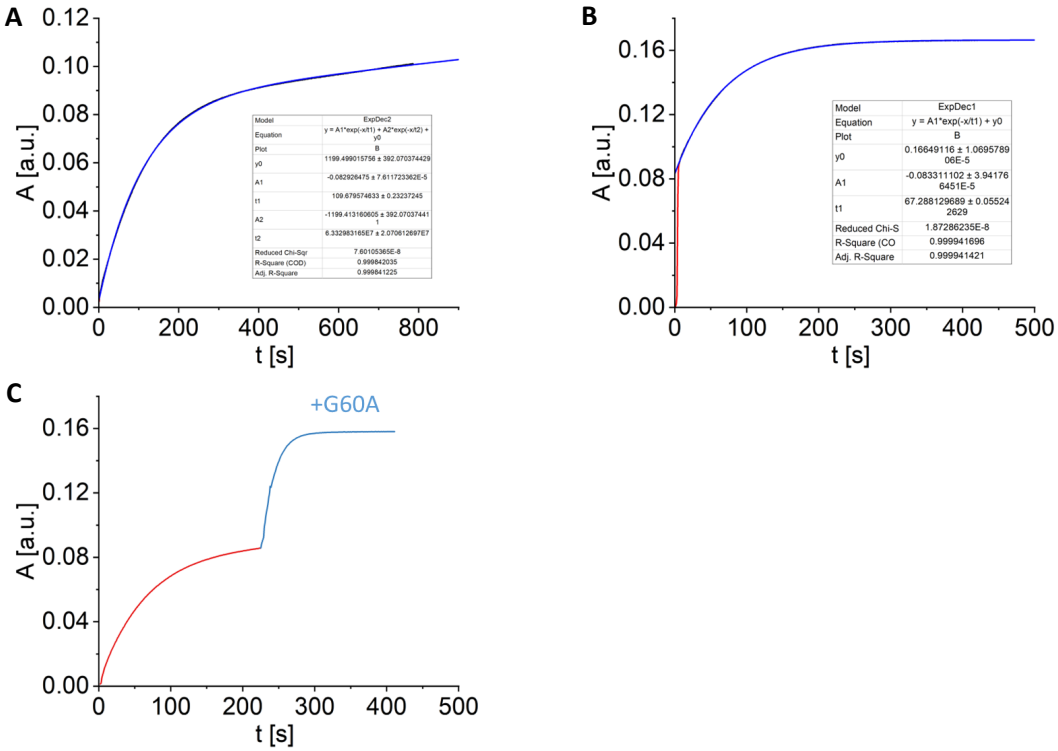

Figure S 80: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_H254G_H257Y
A Time course for the hydrolysis of 10 µM IV-R_P/S_P by 50 nM PTE_I106A_F132A_H254G_H257Y (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_I106A_F132A_H254G_H257Y for the hydrolysis of IV-R_P. **B** Time course for the hydrolysis of 10 µM IV-R_P/S_P by 2 µM 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 µM 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

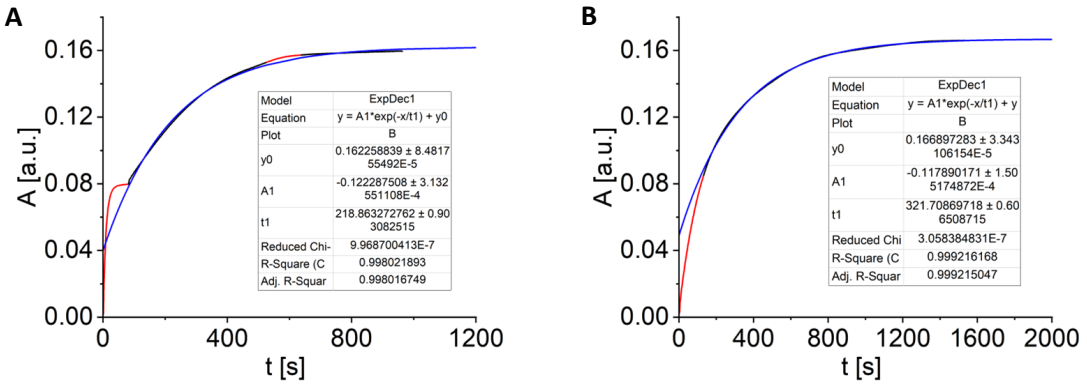

Figure S 81: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_H254G_H257Y

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

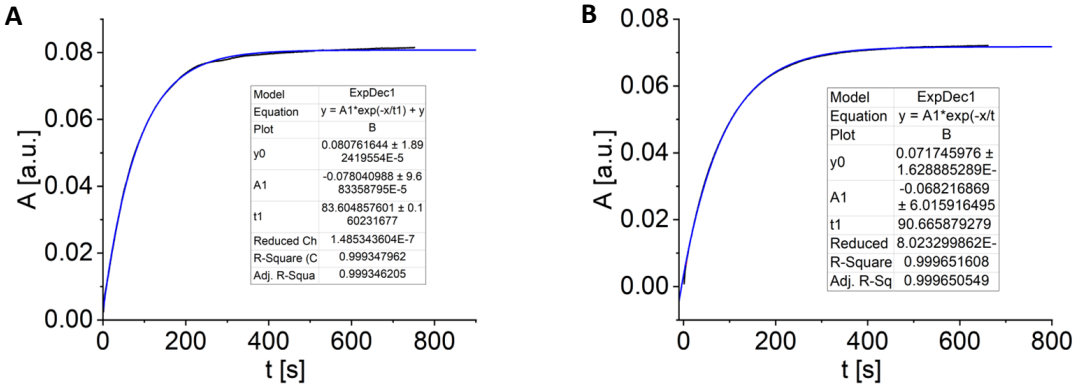

Figure S 82: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_H254G_S308A_H257ONBY before and after illumination

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

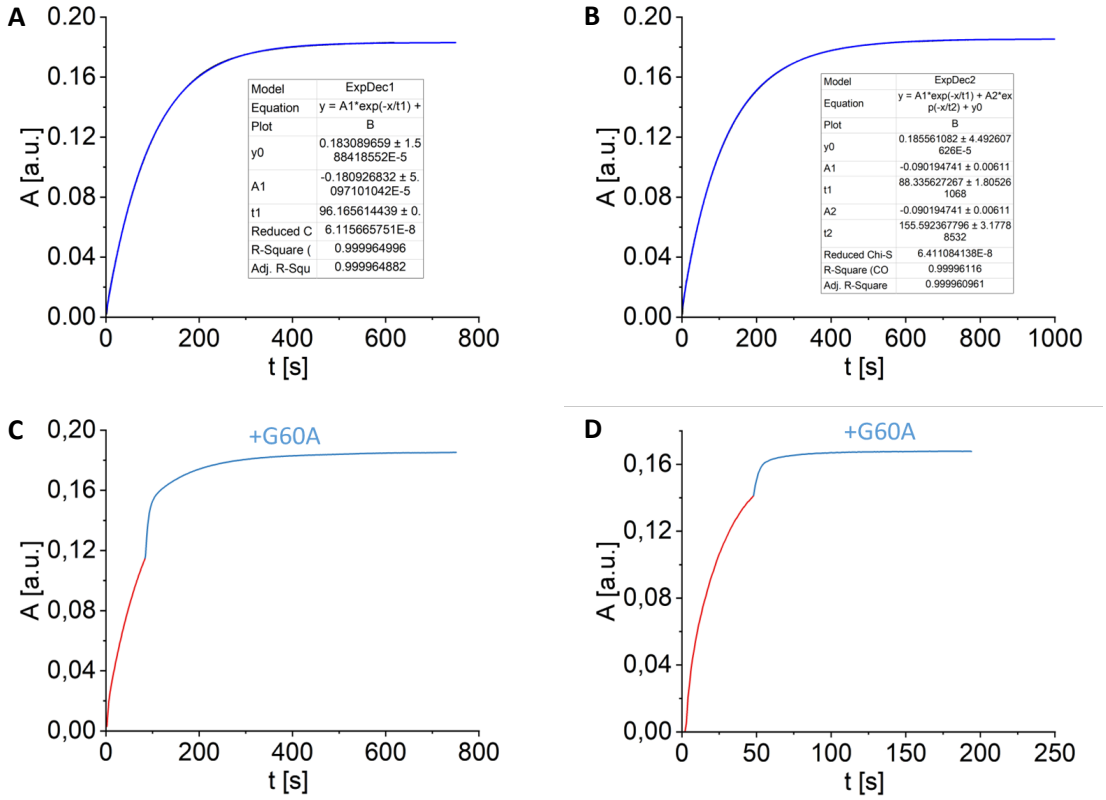

Figure S 83: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_H254G_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 µM 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 µM 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 µM 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 µM 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

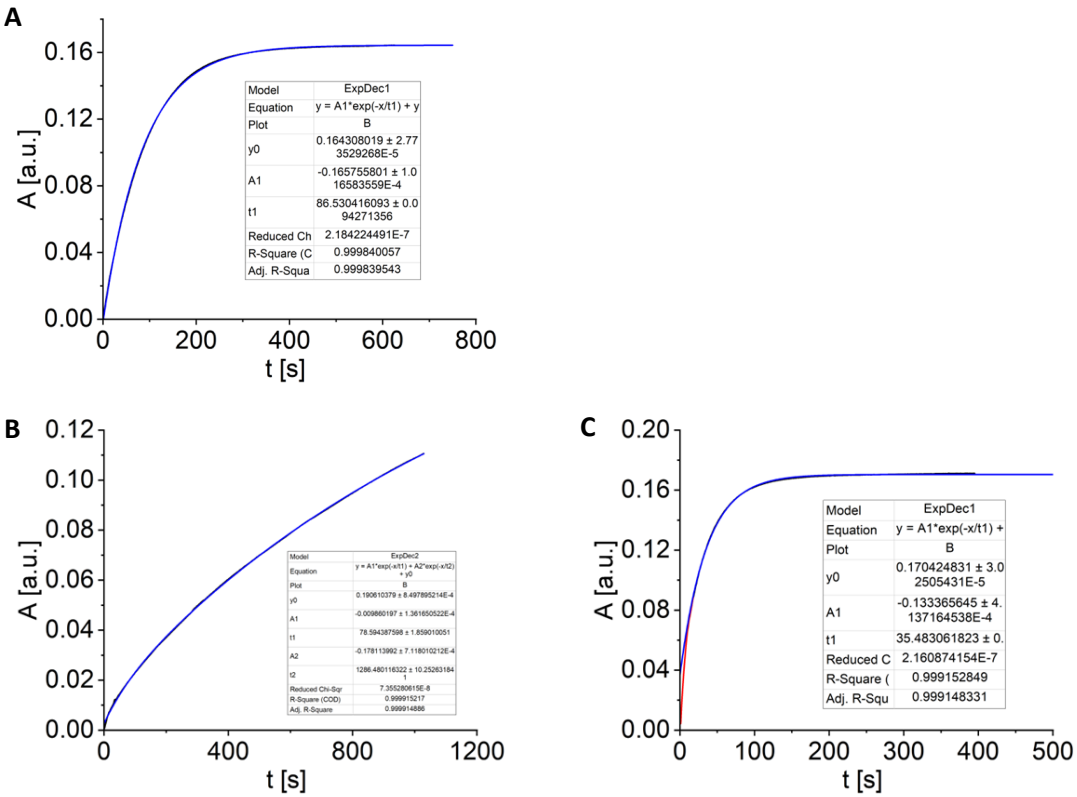

Figure S 84: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_H254G_S308A_H257ONBY before and after illumination

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 (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 after irradiation at 365 nm. Data points corresponding to the hydrolysis of III-R_P (red) were not considered in the fit.

Substrate IV

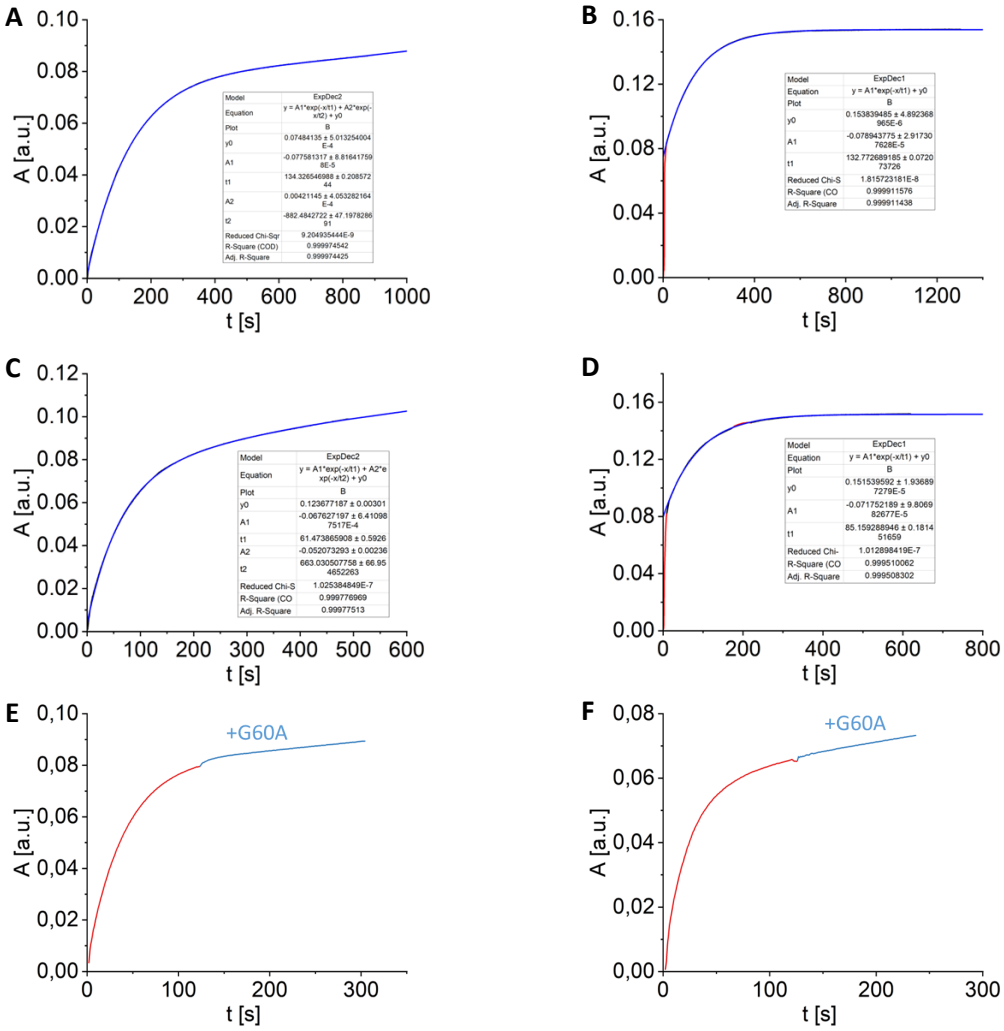

Figure S 85: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_H254G_S308A_H257ONBY before and after illumination

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

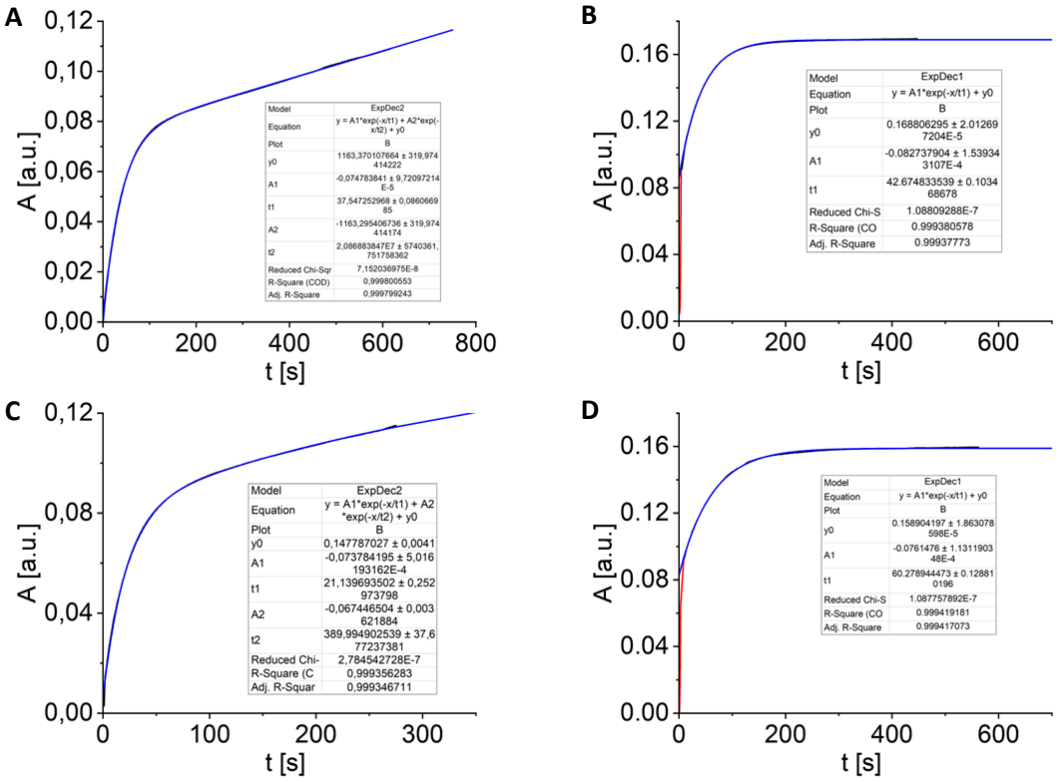

Figure S 86: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_H254G_S308A_H257ONBY before and after illumination

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

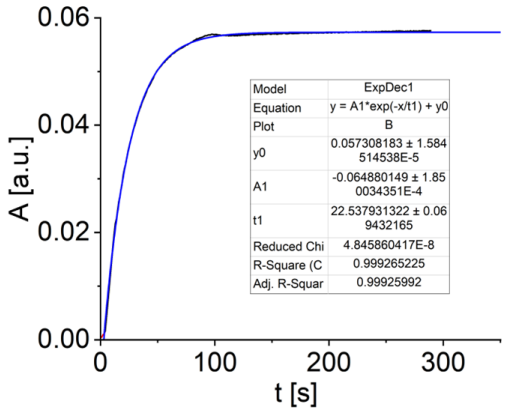

Figure S 87: Enzymatic hydrolysis of substrate I catalyzed by PTE_ I106A_H254G_H257Y_S308A
Time course for the hydrolysis of 5 µM substrate I by addition of 5 nM PTE_I106A_H257Y (black). The data was fitted to a single exponential function.

Substrate II

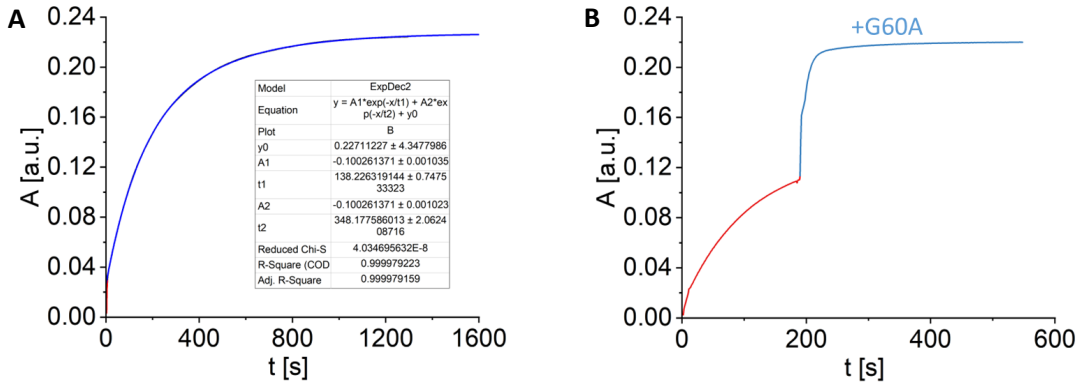

Figure S 88: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_H254G_H257Y_S308A
A Time course for the hydrolysis of 10 µM II- R_P/S_P by 1 nM PTE_I106A_H254G_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency 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 absorption upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed.

Substrate III

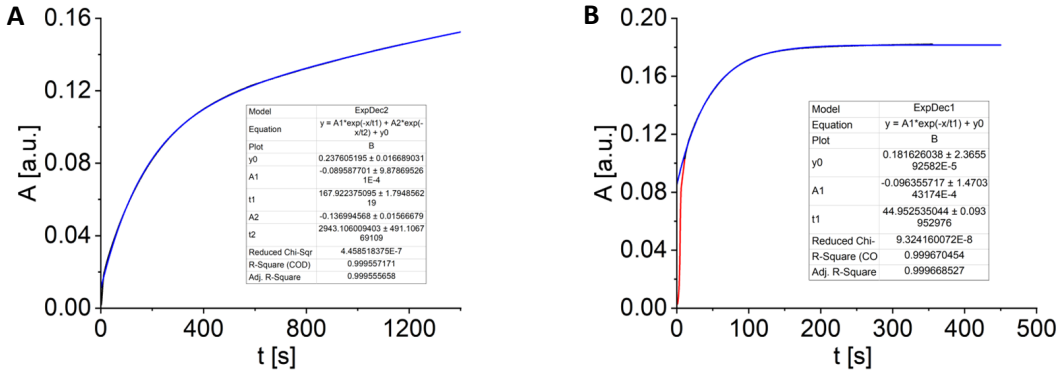

Figure S 89: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_H254G_H257Y_S308A
A Time course for the hydrolysis of 10 µM III-S_P/R_P by 1.5 nM PTE_I106A_H254G_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency 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

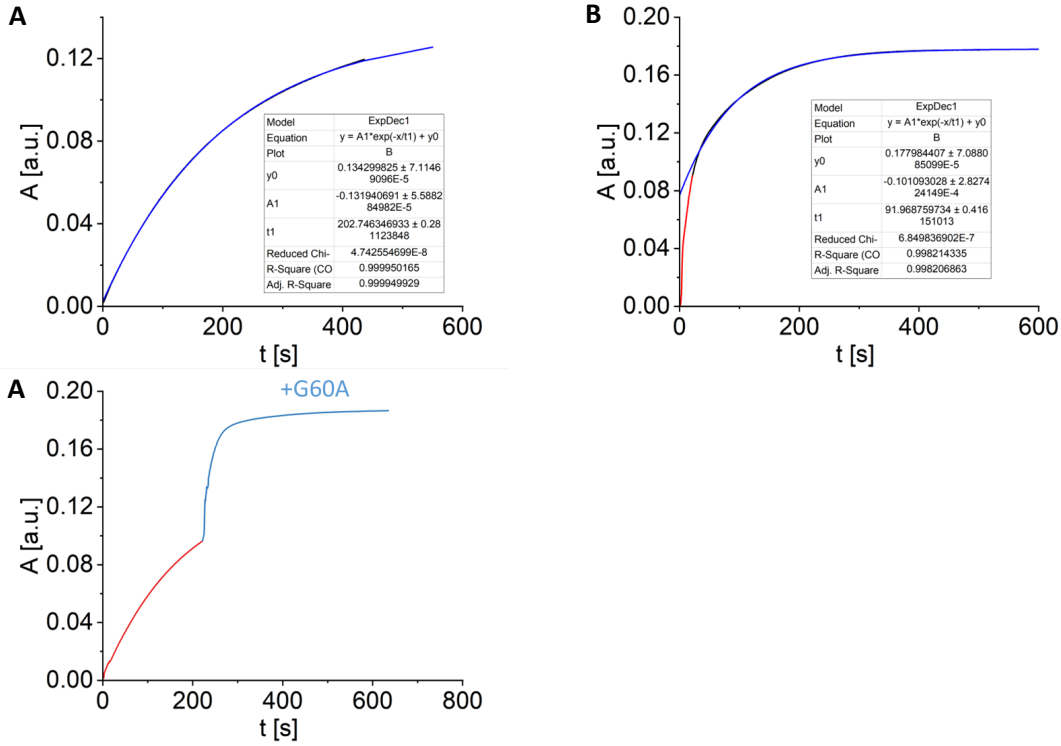

Figure S 90: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_H254G_H257Y_S308A
A Time course for the hydrolysis of 10 µM IV-R_P/S_P by 15 nM PTE_I106A_H254G_H257Y_S308A (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency 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

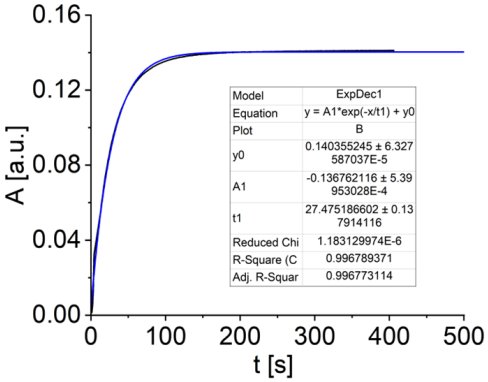

Figure S 91: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_H254G_H257Y_S308A

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

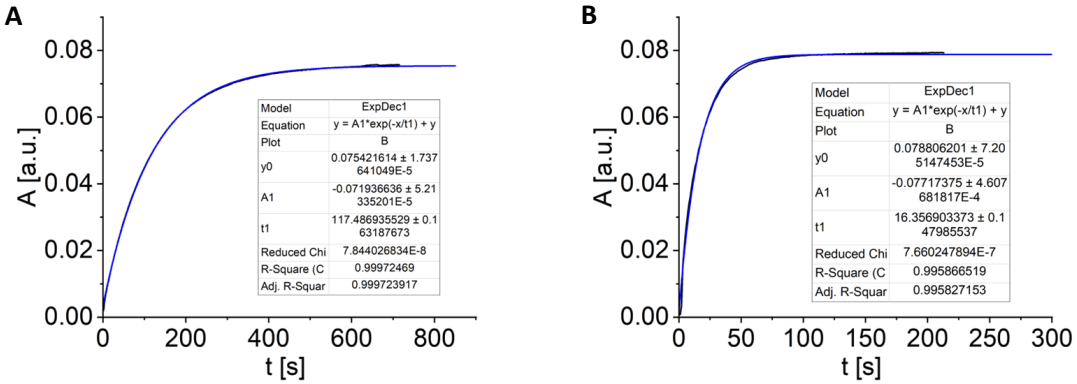

Figure S 92: Enzymatic hydrolysis of substrate I catalyzed by PTE_I106A_F132A_S308A_H257ONBY before and after illumination

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

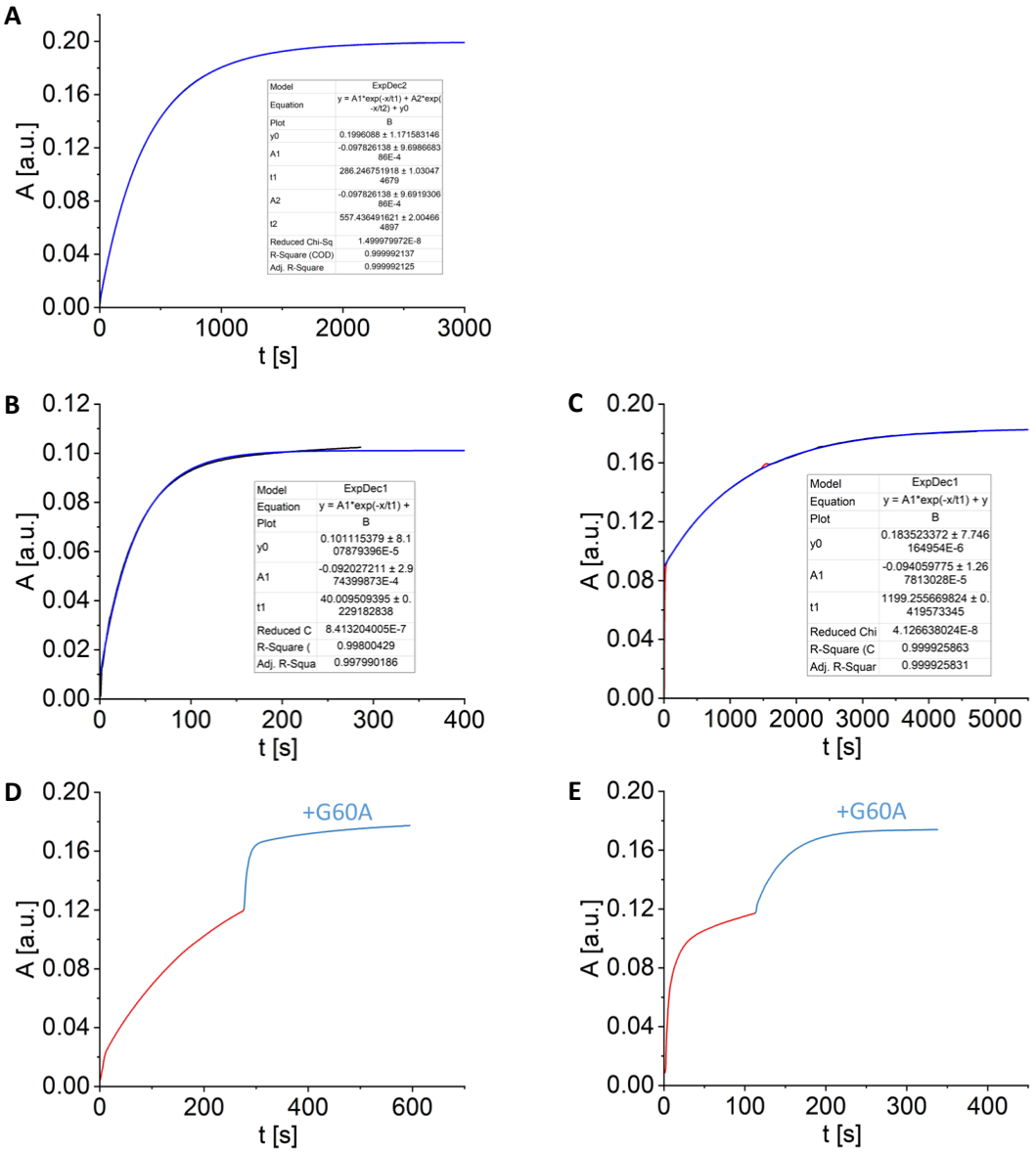

Figure S 93: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F132A_S308A_H257ONBY before and after illumination

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 absorption 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 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

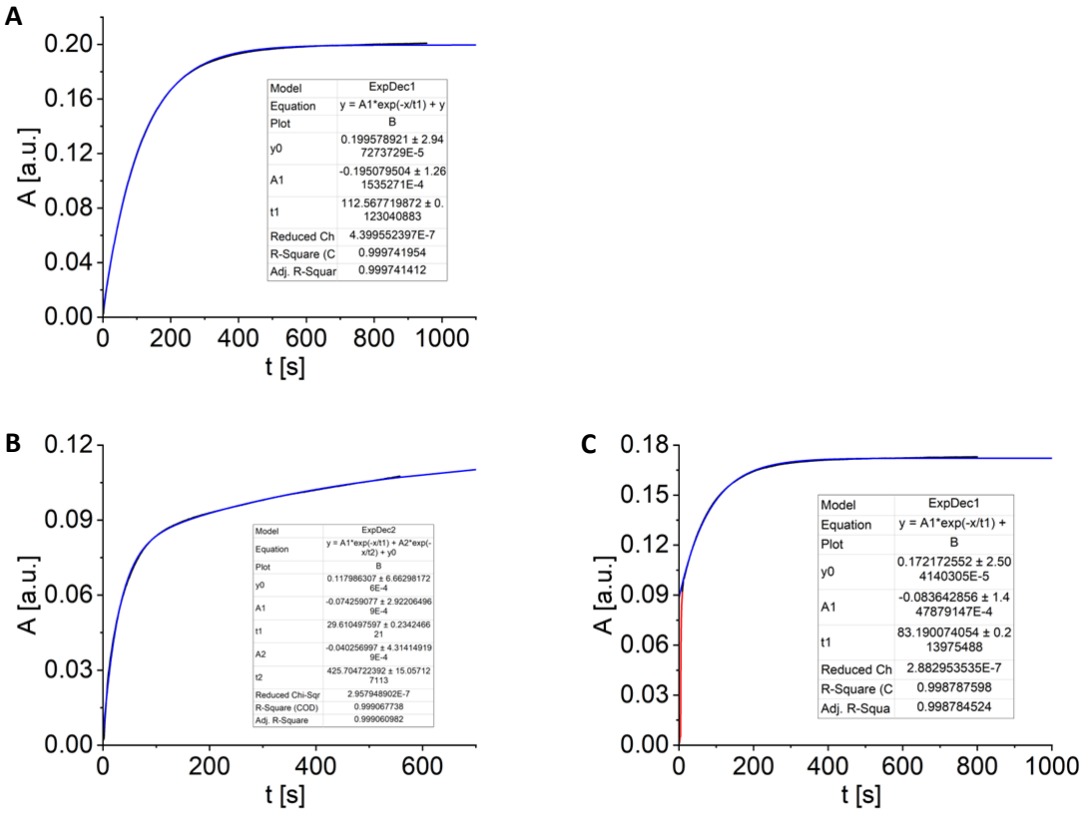

Figure S 94: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_S308A_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 µM 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 µM 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 µM 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

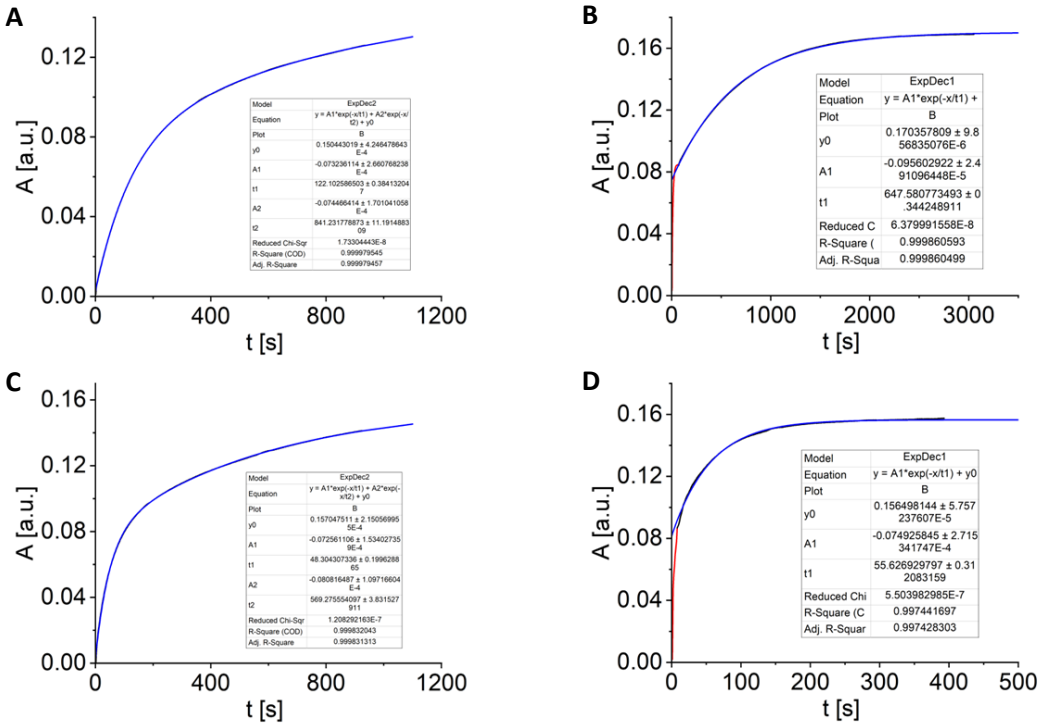

Figure S 95: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_S308A_H257ONBY before and after illumination

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

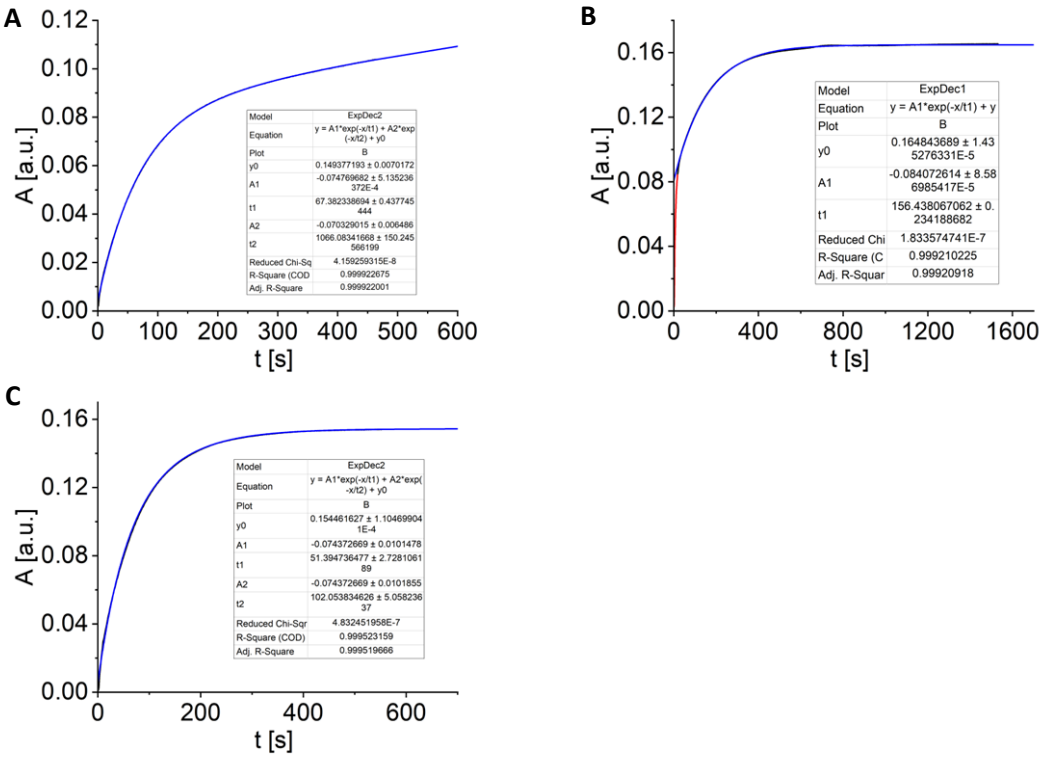

Figure S 96: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_S308A_H257ONBY before and after illumination

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

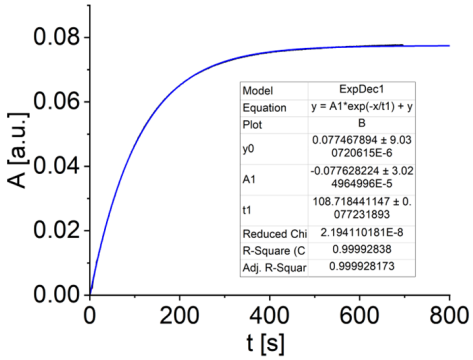

Figure S 97: Enzymatic hydrolysis of substrate I catalyzed by PTE_ I106A_F132A_H257Y_S308A

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

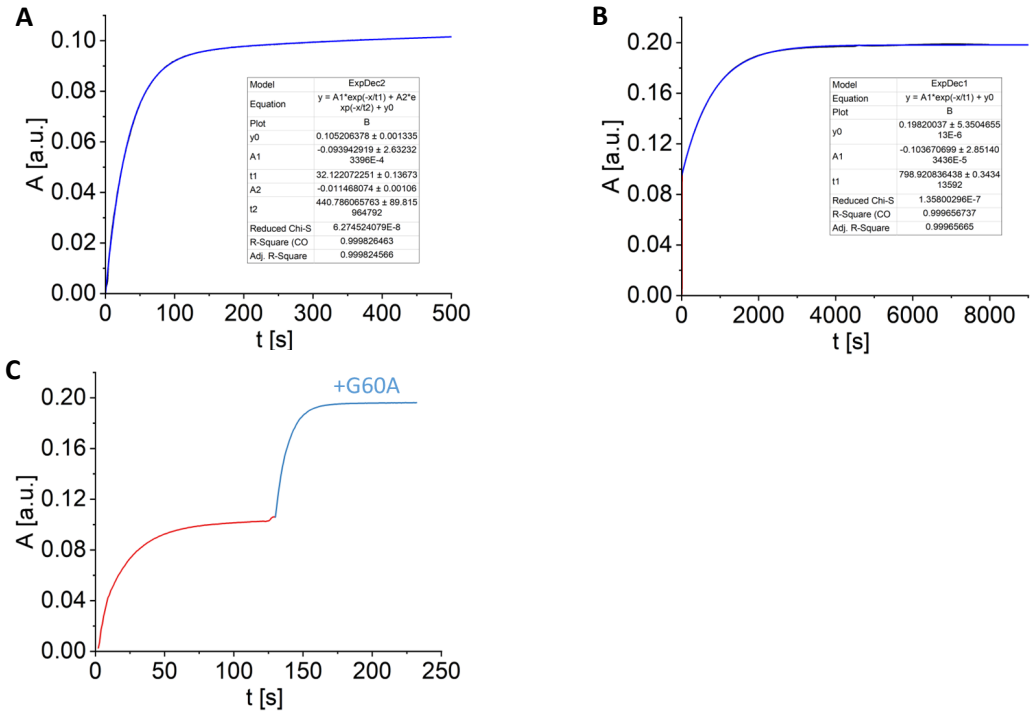

Figure S 98: Enzymatic hydrolysis of substrate II catalyzed by PTE_I106A_F123A_H257Y_S308A

A Time course for the hydrolysis of 10 µM 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 µM 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 µM 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

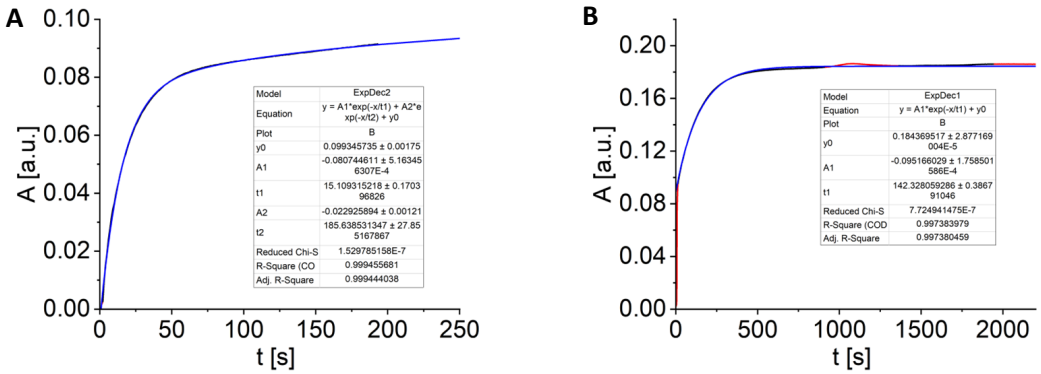

Figure S 99: Enzymatic hydrolysis of substrate III catalyzed by PTE_I106A_F132A_H257Y_S308A

A Time course for the hydrolysis of 10 µM 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 µM 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

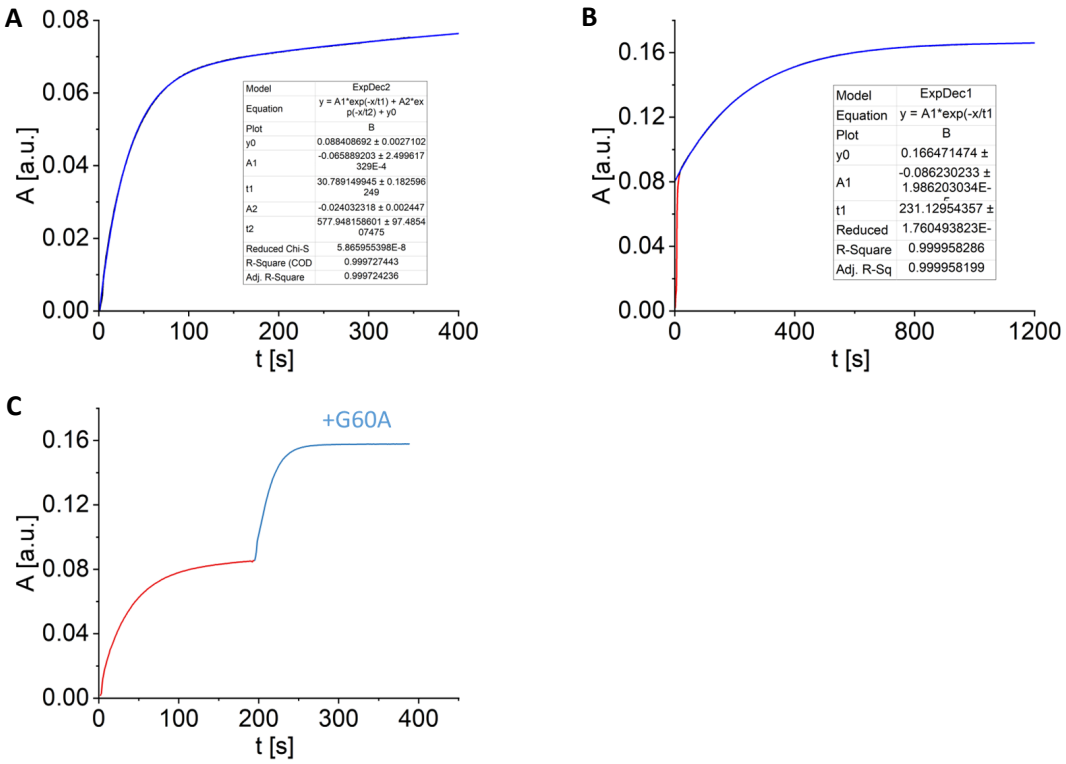

Figure S 100: Enzymatic hydrolysis of substrate IV catalyzed by PTE_I106A_F132A_H257Y_S308A

A Time course for the hydrolysis of 10 µM 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 µM 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 µM 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

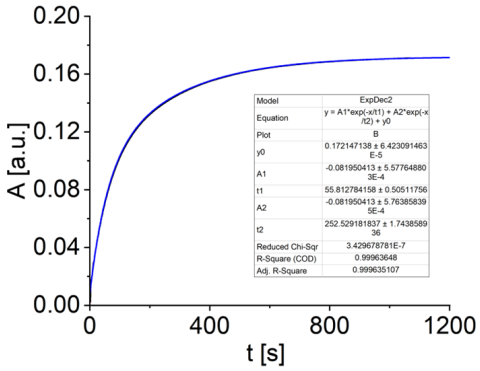

Figure S 101: Enzymatic hydrolysis of substrate V catalyzed by PTE_I106A_F132A_H257Y_S308A

A Time course for the hydrolysis of 10 µM 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

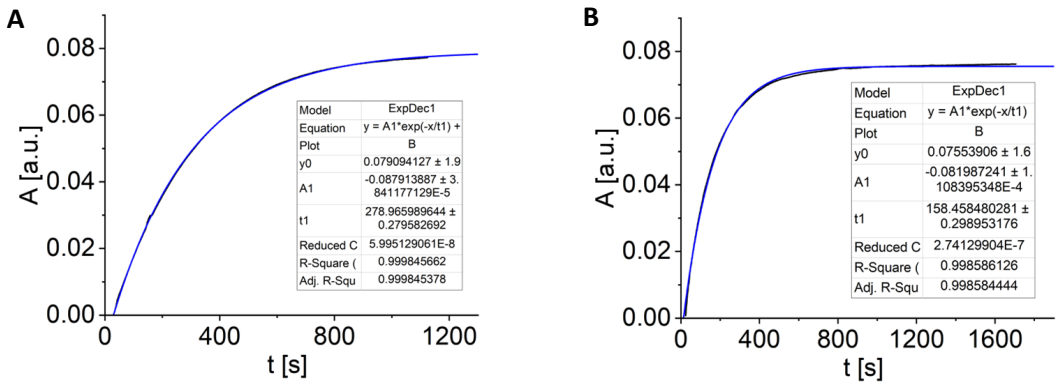

Figure S 102: Enzymatic hydrolysis of substrate I catalyzed by PTE_H254G_L303T_H257ONBY before and after illumination

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

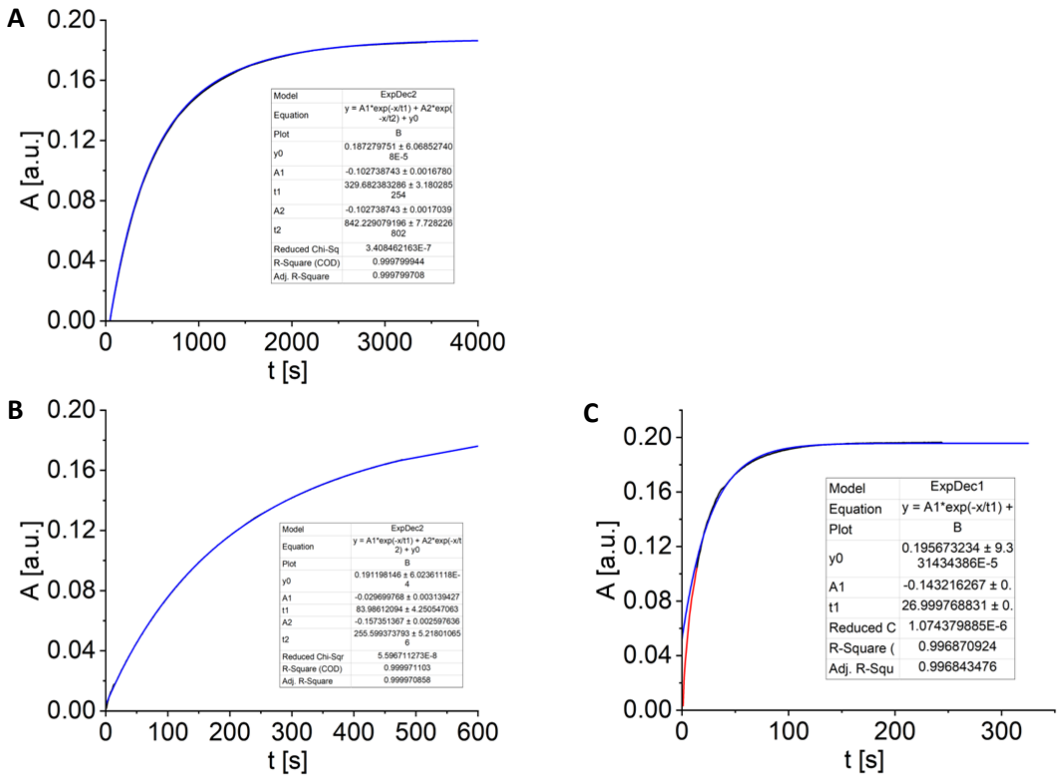

Figure S 103: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254G_L303T_H257ONBY before and after illumination

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

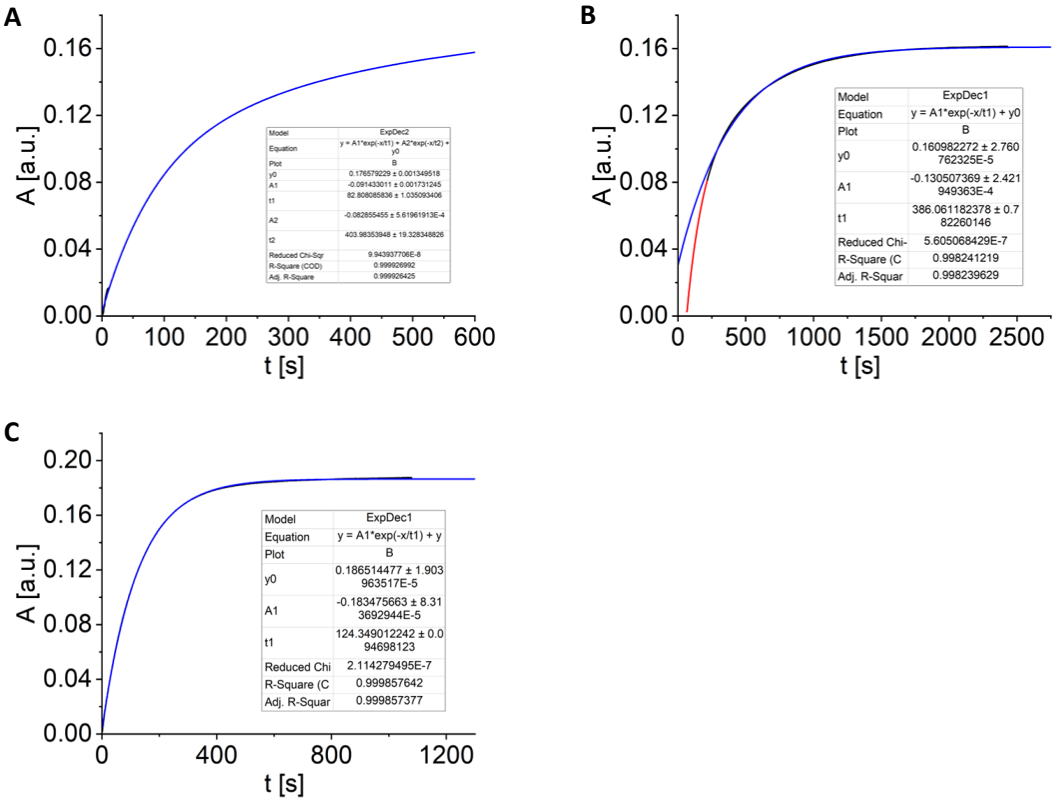

Figure S 104: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254G_L303T_H257ONBY before and after illumination

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

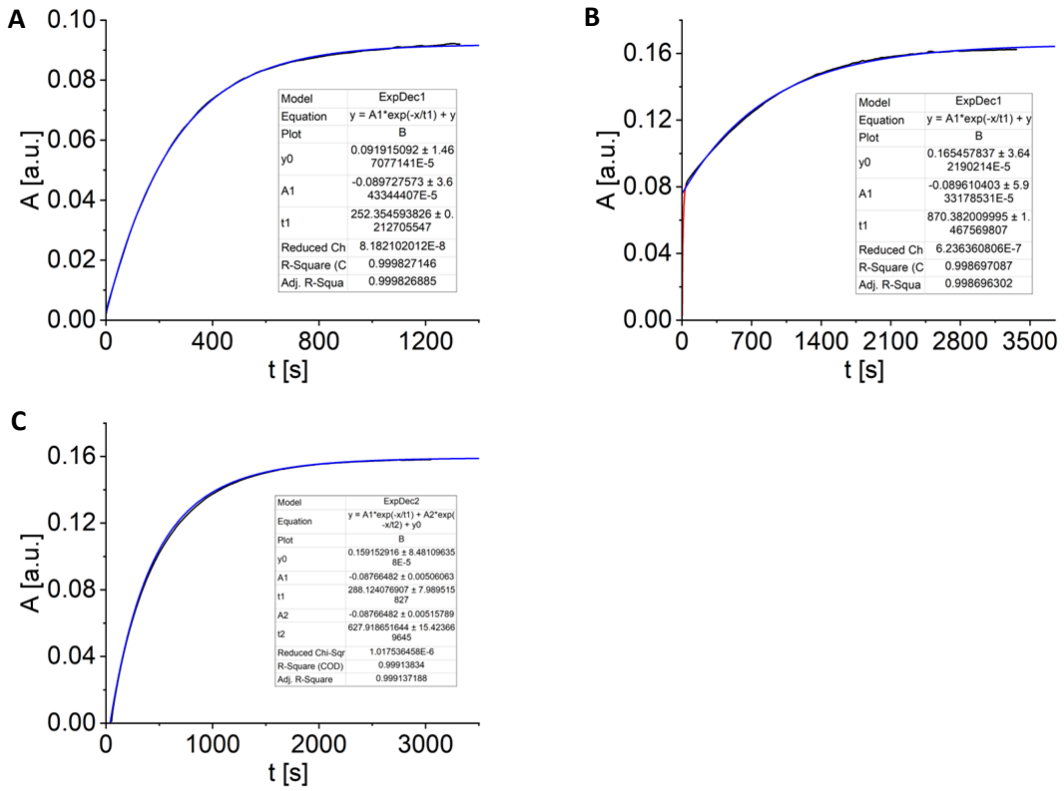

Figure S 105: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254G_L303T_H257ONBY before and after illumination

A Time course for the hydrolysis of 10 µM 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 µM 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_I106A_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 µM 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

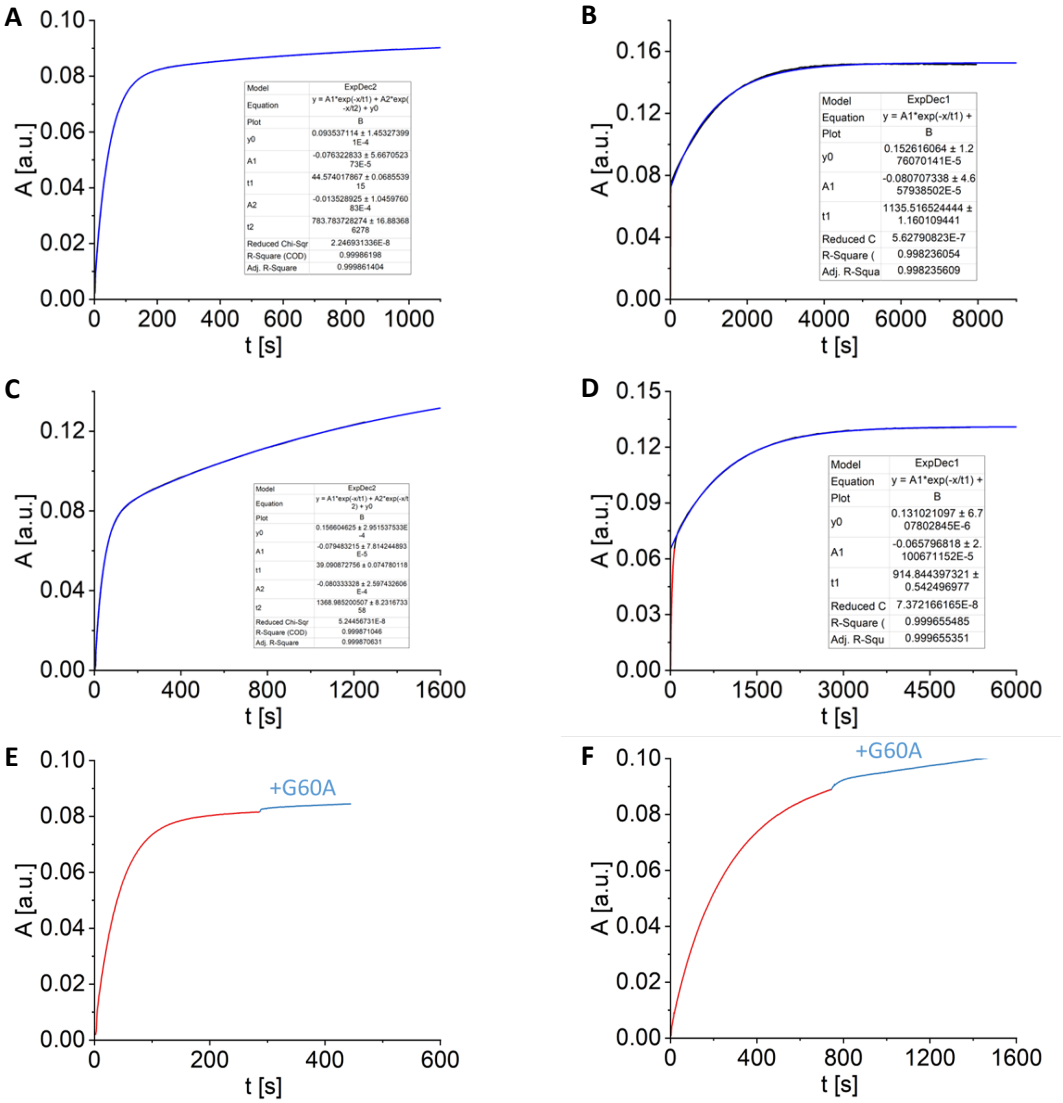

Figure S 106: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254G_L303T_H257ONBY before and after illumination

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

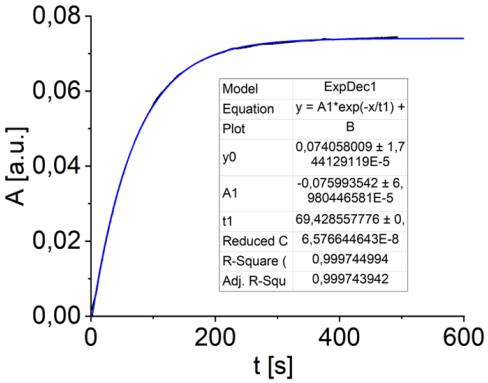

Figure S 107: Enzymatic hydrolysis of substrate I catalyzed by PTE_ H254G_H257Y_L303T
Time course for the hydrolysis of 5 µM substrate I by addition of 5 nM PTE_H254G_H257Y_L303T (black). The data was fitted to a single exponential function.

Substrate II

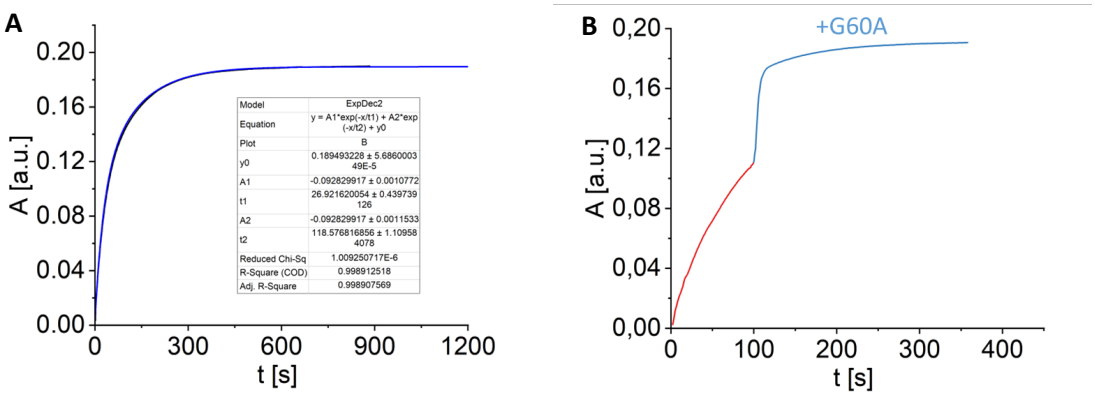

Figure S 108: Enzymatic hydrolysis of substrate II catalyzed by PTE_H254G_H257Y_L303T
A Time course for the hydrolysis of 10 µM II-R_P/S_P by 5 nM PTE_H254G_H257Y_L303T (black). The data was fitted to a double exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_H257Y_L303T 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 absorption upon addition of PTE_G60A indicates that II-R_P is preferentially hydrolyzed.

Substrate III

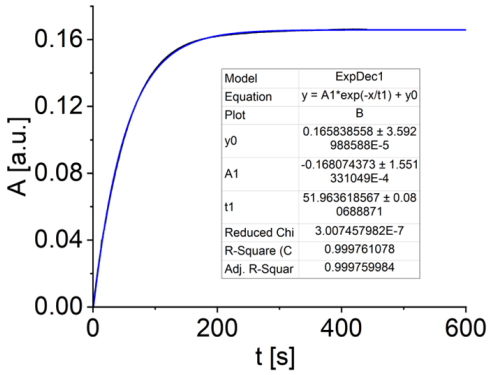

Figure S 109: Enzymatic hydrolysis of substrate III catalyzed by PTE_H254G_H257Y_L303T
A Time course for the hydrolysis of 10 µM III-S_P/R_P by 5 nM PTE_H254G_H257Y_L303T (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_H257Y_L303T for the hydrolysis of III-R_P/S_P.

Substrate IV

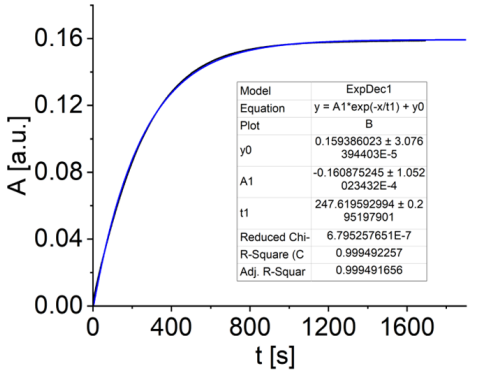

Figure S 110: Enzymatic hydrolysis of substrate IV catalyzed by PTE_H254G_H257Y_L303T
A Time course for the hydrolysis of 10 µM IV-R_P/S_P by 20 nM PTE_H254G_H257Y_L303T (black). The data was fitted to a single exponential function (blue) that was used to determine the catalytic efficiency of PTE_H254G_H257Y_L303T for the hydrolysis of IV-S_P/R_P.

Substrate V

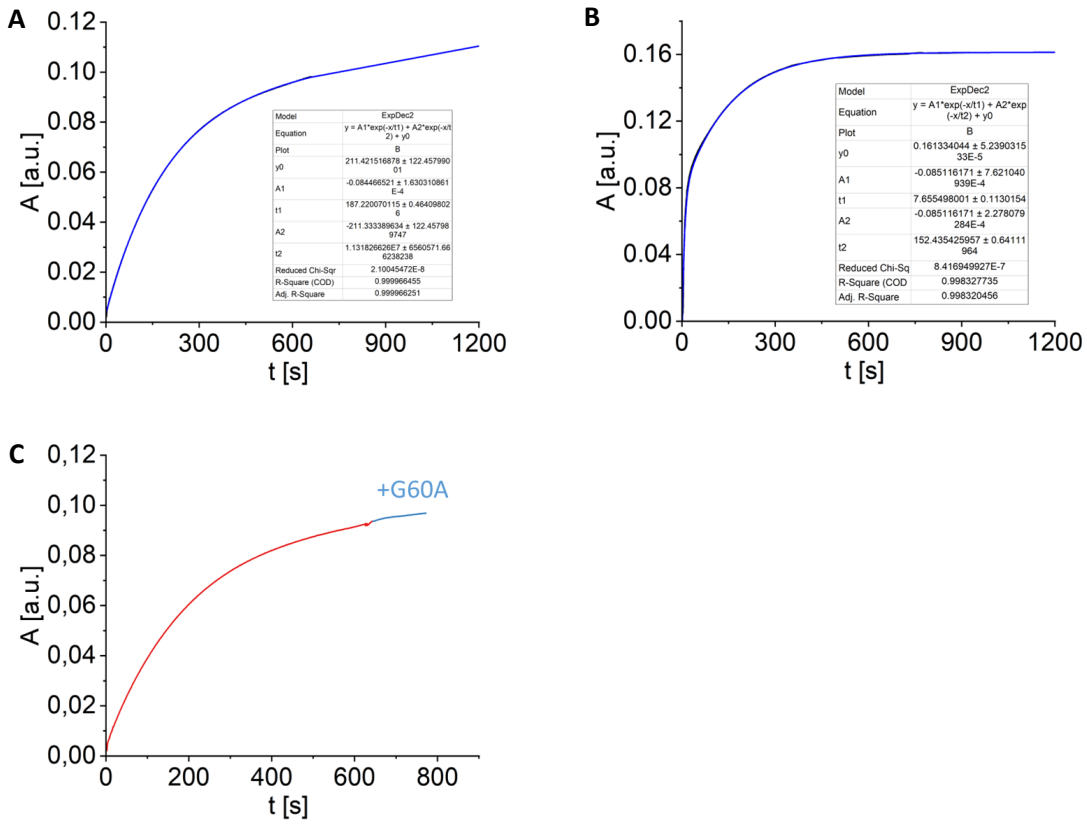

Figure S 111: Enzymatic hydrolysis of substrate V catalyzed by PTE_H254G_H257Y_L303T

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.