Supporting Information

Fusion of a flavin-based fluorescent protein to hydroxynitrile lyase from Arabidopsis thalia improves enzyme stability

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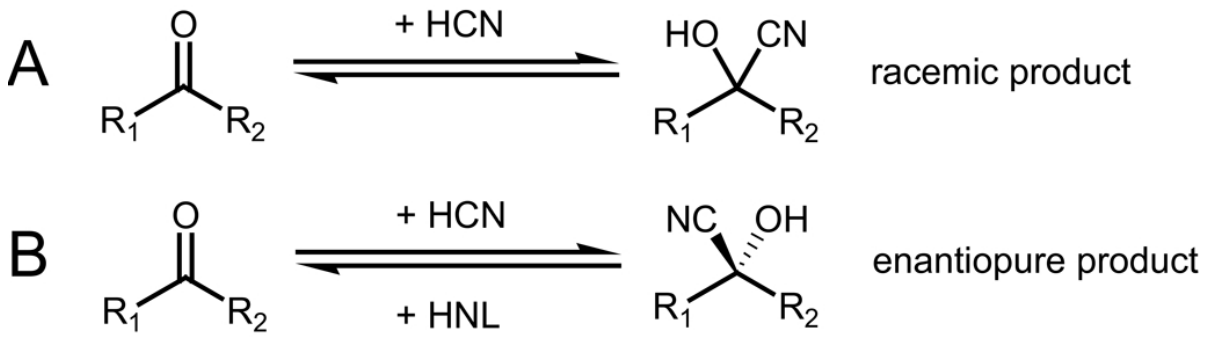
Supplementary Figures and Tables

Supplementary Table 1. Oligonucleotides used in this study		
name	**sequence**[a]	**purpose**
cFbFP_fw	5’-aca gag agc tca tgg cta gtt ttc aat cat ttg gga tac-3’	amplification of cFbFP
cFbFP_rev	5’- gcg ctc gag tta gat atc att ctg aat tcc gac aaa ata c-3’	amplification of cFbFP
nFbFP_fw:	5’-aca gac ata tgg tcg gtg tgg taa tta cag atc ccg-3’	amplification of nFbFP
nFbFP_rev:	5’-ctg tga gct ctg aaa gtg cag taa ttt ccg tga ggg-3’	amplification of nFbFP
cHNL_*Nhe*I_fw	5’-ata tgc tag cat gga gag gaa aca tca ctt cg-3’	amplification of AtHNL for cloning into pETcFbFP
cHNL_blunt_rev	5’-cat ata atc ggt ggc aat agc aga gag aga gtc aaa gag t-3’	amplification of AtHNL for cloning into pETcFbFP
nHNL_blunt_fw	5’- atg gag agg aaa cat cac ttc gtg tta gtt ca-3’	amplification of AtHNL for cloning into pETnFbFP
nHNL_blunt_rev	5’-tta cat ata atc ggt ggc aat agc aga gag aga g-3’	amplification of AtHNL for cloning into pETnFbFP
nYFP_*Nde*I_for	5’-gaa gga gat ata cat atg gtg agc aag ggc gag gag ctg t-3’	First and second round of overlap-extension PCR; amplification of YFP and full-length nYFP-AtHNL
nYFP_GS_rev	5’-ctc cat gct gcc gcc gcc gct gcc gcc gcc gct gcc gcc gcc ctt gta cat ctc gtc cat gcc gag agt gat ccc-3’	First round of overlap-extension PCR; amplification of YFP
nYHNL_GS_for	5’- tac aag ggc ggc ggc agc ggc ggc ggc agc ggc ggc ggc agc atg gag agg aaa cat cac ttc gtg tta gtt cac aac gc-5’	First round of overlap-extension PCR; amplification of YFP
nYHNL_*Not*I _rev	5’- tat ata gcg gcc gca agc ttg tcg act tac ata taa tcg g -3’	First and second round of overlap-extension PCR; amplification of YFP and full-length nYFP-AtHNL
[a] restriction endonuclease cleavage sites used for cloning of the amplified fragments into pET28a are underlined.		

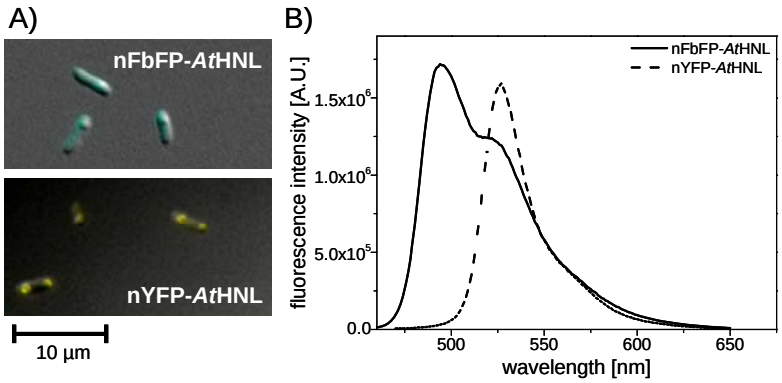
Supplementary Table 2. Native quaternary structure of all constructs used in this study derived from HPLC-SEC analysis.

	***At*HNL**	**cFbFP-*At*HNL**	**nFbFP-*At*HNL**	**nYFP-*At*HNL**
predicted subunit size[a]	29,216	46,360	46,198	59,128
apparent molecular weight [b]	48,000	179,000	85,000	110,000
predominant oligomer [b]	dimer	tetramer	dimer	dimer

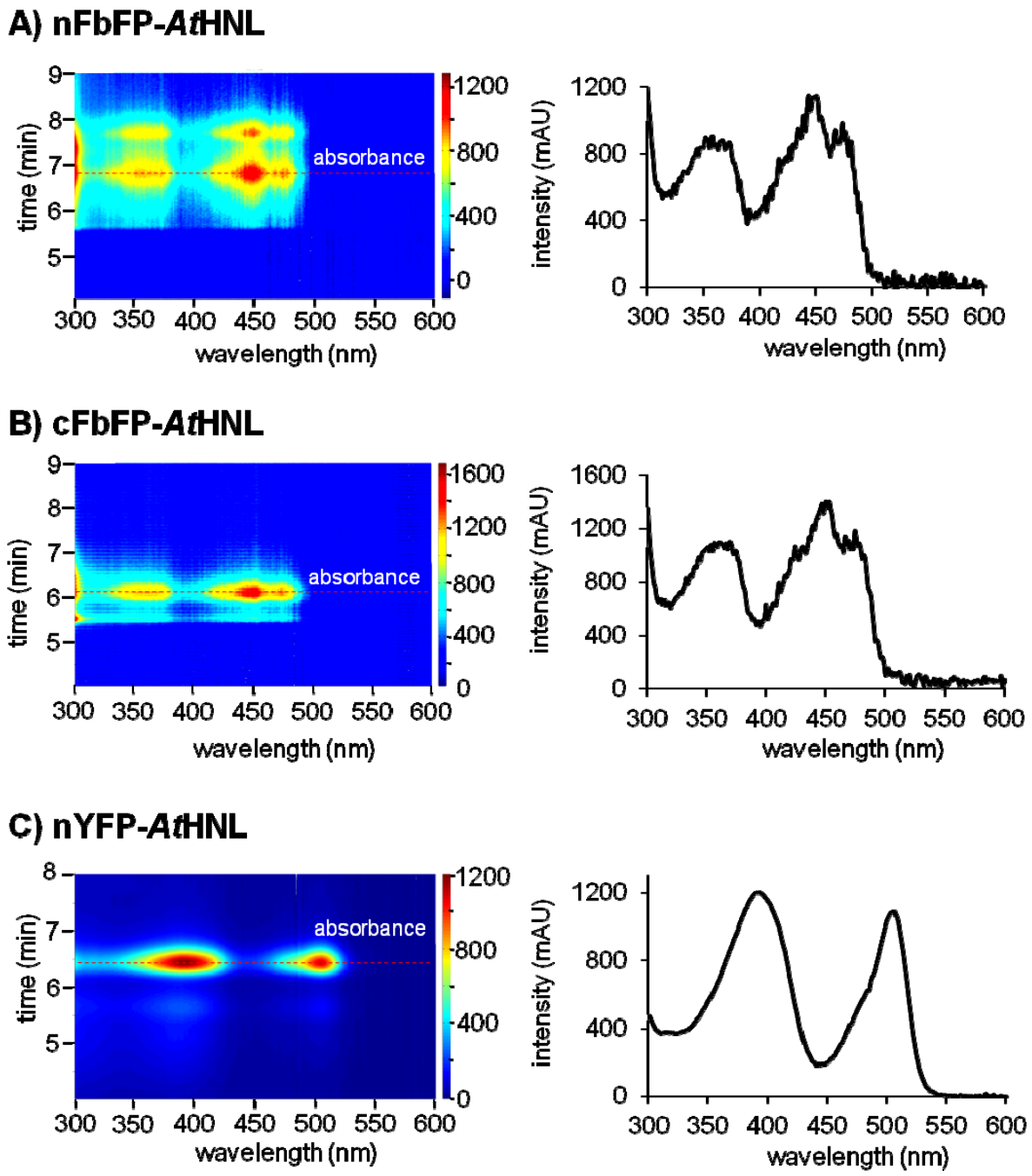
[a] predicted from amino acid sequences using ProtParam (http://web.expasy.org/protparam/)
[b] values are only given for the main peak in Fig. 2, although other oligomeric species are present.



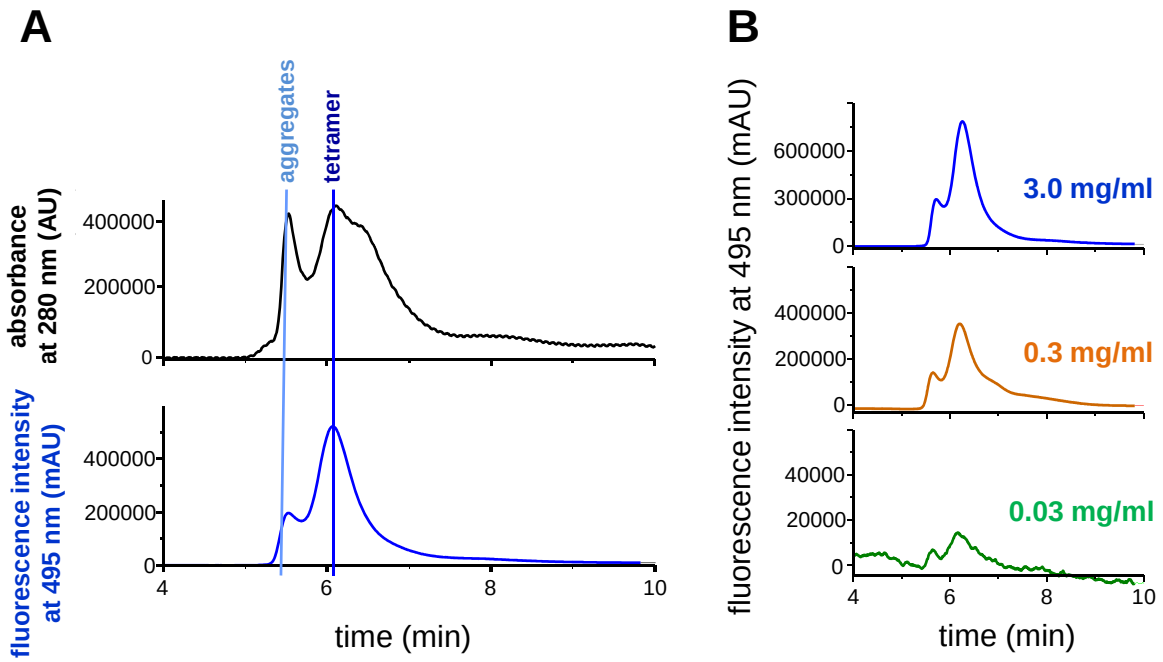
Supplementary Figure 1: pH-dependent non-enzymatic (A) and enzymatic (B) cyanohydrin formation in aqueous media. At low pH values the non-enzymatic route is essentially suppressed.



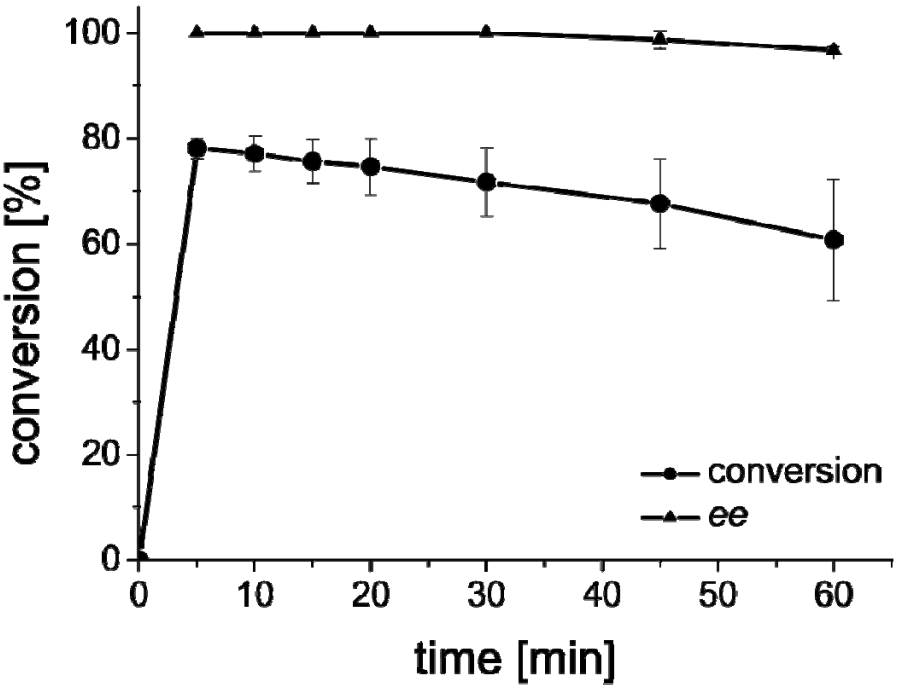
Supplementary Figure 2: A) Microscopic image of fluorescent nFbFP-*At*HNL and nYFP-*At*HNL expressing *E. coli* BL21(DE3) cells. B) FbFP and YFP specific fluorescence emission spectra recorded on whole cells expressing the respective fusion construct. Identical results were obtained with cFbFP-*At*HNL expressing *E. coli* BL21(DE3) cells.



Supplementary Figure 3: UV/Vis Absorbance heat-maps (left) for the relevant part of the HPLC-SEC chromatogram of A) nFbFP-*At*HNL, B) cFbFP-*At*HNL and C) nYFP-*At*HNL. The maximum of the respective elution peak is marked by a broked red line (corresponding to maximum absorbance at 280 nm). Absorbance spectra (right) were obtained from the respective heat-map data matrix at the retention time corresponding to the elution peak maximum (broken red line in heat-map). All in the manuscript discussed elution peaks do contain the respective chromophore. This allows unequivocal identification of the corresponding dimeric/tetrameric species as the target fusion protein.



Supplementary Figure 4: A) Comparison of the HPLC-SEC elution profile of cFbFP-*At*HNL followed by UV/Vis absorbance detection at 280 nm (upper panel, black line) and fluorescence detection (lower panel, blue line). FbFP specific excitation was carried out at 450 nm. Fluorescence emission was followed at 495 nm. B) Comparison of the elution profiles of cFbFP-*At*HNL for samples of the indicated concentration. Elution was followed by fluorescence detection of the fluorescent reporter-protein tag (FbFP, excitation 450 nm, emission 495 nm). At a protein concentration of 0.03 mg/mL which is two-times lower than the average enzyme concentration used in the assay (0.06 mg/mL), no detectable dissociation of the tetrameric cFbFP-*At*HNL species does occur. Similar results were obtained for the nFbFP-*At*HNL and the nYFP-*At*HNL fusion protein (data not shown).



Supplementary Figure 5: Conversion of 2-chlorobenzaldehyde and HCN to (*R*)-chloromandelonitrile by cFbFP-*At*HNL in an aqueous-organic two-phase system containing methyl *tert*-butylether and 50 mM acetate buffer (pH 5). The organic phase contained the substrates (1 mL, 1.5-2 M HCN, 0.5 mmol benzaldehyde). Enzymatic conversion, using 4 mg/mL enzyme, was followed using chiral gas chromatography. Reactions were performed at 25 °C. Error bars represent the standard deviation derived from two independent measurements.

Supplementary Materials and Methods

Spectrofluorimetric analyses. Fluorescence measurements were performed by using thermostated (25 °C) T-format Fluorolog-3 spectrofluorimeter (Horiba, Kyoto, Japan) equipped with double-grating excitation and emission monochromators and a 450 W Xenon CW lamp as excitation source. FbFP and YFP fluorescence emission spectra were recorded from diluted whole cell samples in 10 mm quartz cuvettes. *E. coli* BL21(DE3) cells expressing the respective fluorescent fusion construct were diluted in 50 mM sodium phosphate buffer to an OD_{600nm} of 0.1. FbFP emission spectra (470 nm to 650 nm) were recorded after excititation at 450 nm. YFP samples were excited at 450 nm and emission spectra were recorded from 470 nm to 650 nm. Absorbance measurements were carried out using a UV/Vis spectrophotometer UV 1800 (Shimadzu, Kyoto Japan).

Microscopic analyses 3 µL of the nFbFP-*At*HNL or nYFP-*At*HNL expressing *E. coli* BL21(DE3) (OD_{600nm} = 1) cell suspension was immobilized on a glass slide covered with a 1 % agarose pad of uniform thickness. The sample was covered with a cover glass slide and placed on a fully motorized inverted microscope (Nikon Eclipse Ti, Nikon Instruments Inc., Melville, NY, USA). A Xenon fluorescence light source was used for fluorescence excitation. Standard filters for the proper excitation and detection of the FbFP reporter (eCFP excitation: 426 nm - 446 nm and emission lines: 460 nm - 500 nm; YFP excitation: 490 nm – 510 nm; emission: 520 nm – 550 nm) were used. DIC microscopy images as well as fluorescence images were captured and analyzed using the Nikon NIS Elements AR software package.