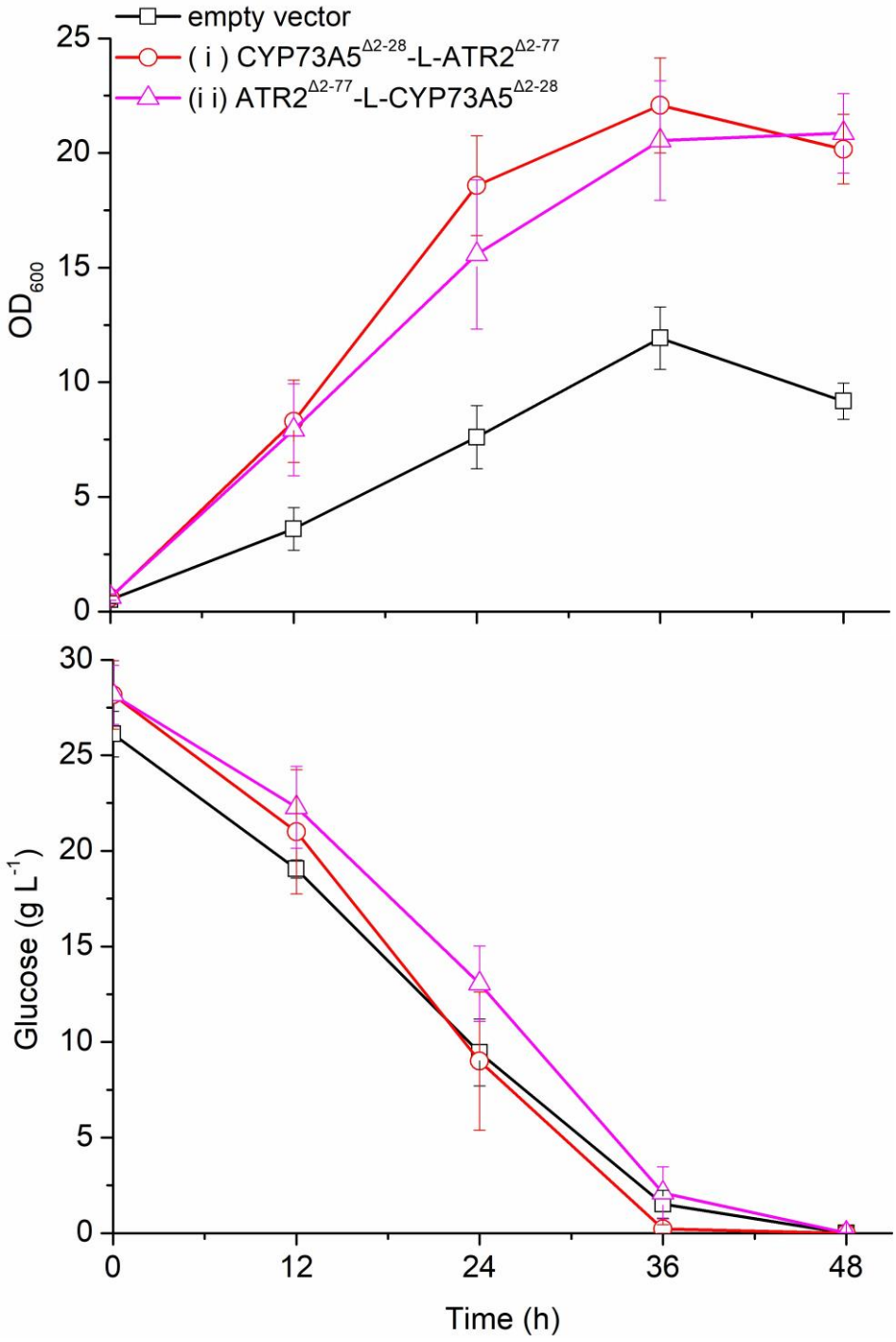
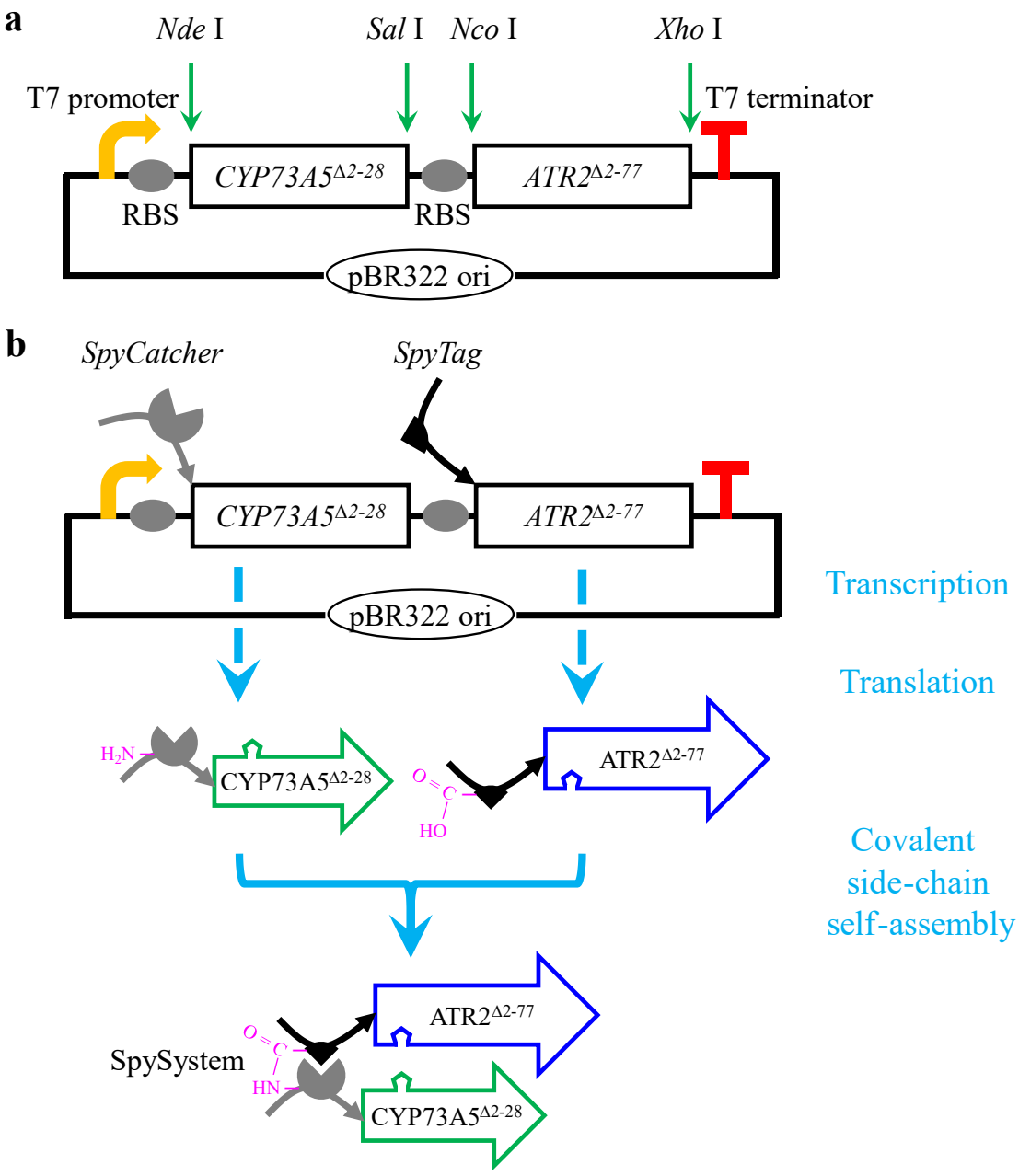
# Tuning architectural organization of eukaryotic P450 system to boost bioproduction in *Escherichia coli*

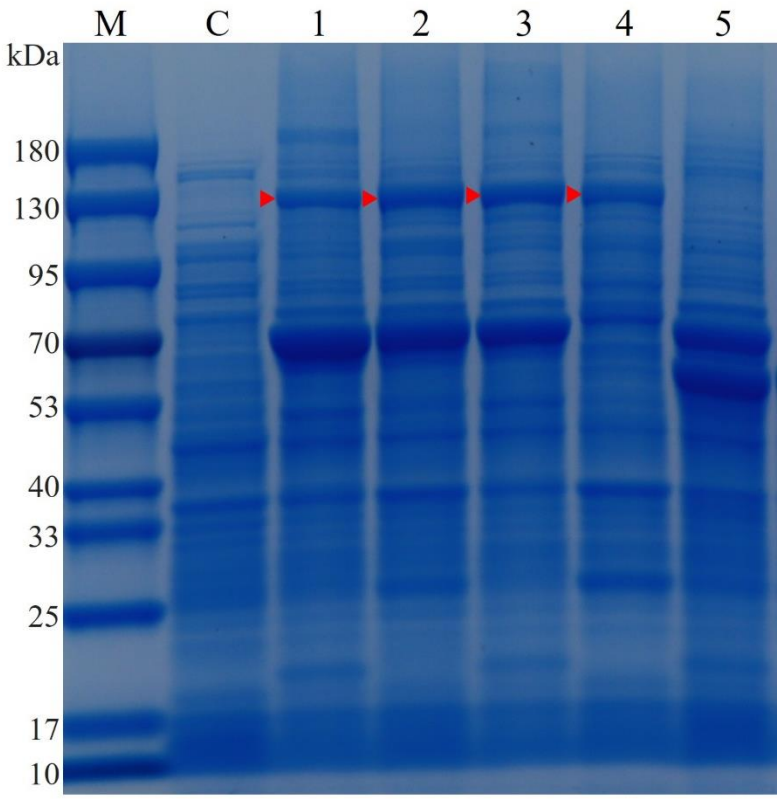
Li *et al.*




**Supplementary Fig. 1. Cell growth ($OD_{600}$) and glucose consumption of *A. thaliana* PAL1-expressed *E. coli* strains.** Empty vector (black), the tandem fused chimera (i) CYP73A5$^{\Delta 2\text{-}28}$-L-ATR2$^{\Delta 2\text{-}77}$ (red), or the tandem fused chimera (ii) ATR2$^{\Delta 2\text{-}77}$-L-CYP73A5$^{\Delta 2\text{-}28}$ (magenta). The error bars represent the standard error of the mean from n = 8 independent experiments of each strain. Source data are provided as a Source Data file.




**Supplementary Fig. 2. Modular organization of CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$ based on self-assembled peptide bio-machinery.** (**a**) The genes encoding CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$ were co-expressed in a modular manner as a polycistron driven by an IPTG-induced T7 promoter. The unique cleavage sites of restriction endonucleases at the 5'- and 3'-termini of both genes are indicated. (**b**) CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$ were respectively fused in frame with SpyCatcher peptide and SpyTag peptide at each N-terminus. The co-expressed proteins, SpyCatcherCYP73A5$^{\Delta 2\text{-}28}$ and SpyTagATR2$^{\Delta 2\text{-}77}$, form a covalent heterodimer post-translationally due to the covalent side chain self-assembly of the aspartate-lysine peptide ligation system SpySystem[1].




**Supplementary Fig. 3. Detection of SpySystem-linked heterodimers by SDS-PAGE.** Lane M, protein marker with corresponding molecular weight (MW) on the left side; Lane C, sample of the induced BL21(DE3) cells with the empty vector; Lane 1, sample of the induced JIB1925 cells with CYP73A5$^{\Delta 2\text{-}28}$SpyCatcher and SpyTagATR2$^{\Delta 2\text{-}77}$, of which the band about 139.7 KDa is the covalent Heterodimer (I); Lane 2, sample of the induced JIB1926 cells with SpyCatcherCYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$SpyTag, of which the band about 139.7 KDa is the covalent Heterodimer (II); Lane 3, sample of the induced JIB1927 cells with CYP73A5$^{\Delta 2\text{-}28}$SpyCatcher and ATR2$^{\Delta 2\text{-}77}$SpyTag, of which the band about 139.7 KDa is the covalent Heterodimer (III); Lane 4, sample of the induced JIB1928 cells with SpyCatcherCYP73A5$^{\Delta 2\text{-}28}$ and SpyTagATR2$^{\Delta 2\text{-}77}$, of which the band about 139.7 KDa is the covalent Heterodimer (IV); Lane 5, sample of the induced JIB1929 cells with CYP73A5$^{\Delta 2\text{-}28}$ (Calculated MW = 54.9 KDa) and ATR2$^{\Delta 2\text{-}77}$ (Calculated MW = 71.0 KDa). The red arrows indicate the target SpySystem-linked heterodimers in lane 1, 2, 3 and 4. Source data are provided as a Source Data file.

CYP73A5$^{\Delta 2-28}$SpyCatcher:

```
MKKLKLPPGP IPIPIFGNWL QVGDDLNHRN LVDYAKKFGD LFLLRMGQRN LVVVSSPDLT   60
KEVLLTQGVE FGSRTRNVVF DIFTGKGQDM VFTVYGEHWR KMRRIMTVPF FTNKVVQQNR  120
EGWEFEAASV VEDVKKNPDS ATKGIVLRKR LQLMMYNNMF RIMFDRRFES EDDPLFLRLK  180
ALNGERSRLA QSFEYNYGDF IPILRPFLRG YLKICQDVKD RRIALFKKYF VDERKQIASS  240
KPTGSEGLKC AIDHILEAEQ KGEINEDNVL YIVENINVAA IETTLWSIEW GIAELVNHPE  300
IQSKLRNELD TVLGPGVQVT EPDLHKLPYL QAVVKETLRL RMAIPLLVPH MNLHDAKLAG  360
YDIPAESKIL VNAWWLANNP NSWKKPEEFR PERFFEEESH VEANGNDFRY VPFGVGRRSC  420
PGIILALPIL GITIGRMVQN FELLPPPGQS KVDTSEKGGQ FSLHILNHSI IVMKPRNCGA  480
MVDTLSGLSS EQGQSGDMTI EEDSATHIKF SKRDEDGKEL AGATMELRDS SGKTISTWIS  540
DGQVKDFYLY PGKYTFVETA APDGYEVATA ITFTVNEQGQ VTVNGKATKG DAHI        594
```

SpyTagATR2$^{\Delta 2-77}$:

```
MAHIVMVDAY KPTKGNSKRV EPLKPLVIKP REEEIDDGRK KVTIFFGTQT GTAEGFAKAL   60
GEEAKARYEK TRFKIVDLDD YAADDDEYEE KLKKEDVAFF FLATYGDGEP TDNAARFYKW  120
FTEGNDRGEW LKNLKYGVFG LGNRQYEHFN KVAKVVDDIL VEQGAQRLVQ VGLGDDDQCI  180
EDDFTAWREA LWPELDTILR EEGDTAVATP YTAAVLEYRV SIHDSEDAKF NDINMANGNG  240
YTVFDAQHPY KANVAVKREL HTPESDRSCI HLEFDIAGSG LTYETGDHVG VLCDNLSETV  300
DEALRLLDMS PDTYFSLHAE KEDGTPISSS LPPPFPPCNL RTALTRYACL LSSPKKSALV  360
ALAAHASDPT EAERLKHLAS PAGKDEYSKW VVESQRSLLE VMAEFPSAKP PLGVFFAGVA  420
PRLQPRFYSI SSSPKIAETR IHVTCALVYE KMPTGRIHKG VCSTWMKNAV PYEKSENCSS  480
APIFVRQSNF KLPSDSKVPI IMIGPGTGLA PFRGFLQERL ALVESGVELG PSVLFFGCRN  540
RRMDFIYEEE LQRFVESGAL AELSVAFSRE GPTKEYVQHK MMDKASDIWN MISQGAYLYV  600
CGDAKGMARD VHRSLHTIAQ EQGSMDSTKA EGFVKNLQTS GRYLRDVW              648
```

**Supplementary Fig. 4. Protein identification of SpySystem-linked CYP73A5-ATR2 heterodimer (I).** The target band indicated in Supplementary Fig. 3 lane 1 was cut off from the gel, cleaved by trypsin, and then subjected to LC-MS/MS analysis. The matched peptides in the sample were highlighted, covering 78.47% of the full-length amino acid sequence of heterodimer (I).

SpyCatcherCYP73A5$^{\Delta 2-28}$:

```
MGAMVDTLSG LSSEQGQSGD MTIEEDSATH IKFSKRDEDG KELAGATMEL RDSSGKTIST      60
WISDGQVKDF YLYPGKYTFV ETAAPDGYEV ATAITFTVNE QGQVTVNGKA TKGDAHIKKL     120
KLPPGPIPIP IFGNWLQVGD DLNHRNLVDY AKKFGDLFLL RMGQRNLVVV SSPDLTKEVL     180
LTQGVEFGSR TRNVVFDIFT GKGQDMVFTV YGEHWRKMRR IMTVPFFTNK VVQQNREGWE     240
FEAASVVEDV KKNPDSATKG IVLRKRLQLM MYNNMFRIMF DRRFESEDDP LFLRLKALNG     300
ERSRLAQSFE YNYGDFIPIL RPFLRGYLKI CQDVKDRRIA LFKKYFVDER KQIASSKPTG     360
SEGLKCAIDH ILEAEQKGEI NEDNVLYIVE NINVAAIETT LWSIEWGIAE LVNHPEIQSK     420
LRNELDTVLG PGVQVTEPDL HKLPYLQAVV KETLRLRMAI PLLVPHMNLH DAKLAGYDIP     480
AESKILVNAW WLANNPNSWK KPEEFRPERF FEEESHVEAN GNDFRYVPFG VGRRSCPGII     540
LALPILGITI GRMVQNFELL PPPGQSKVDT SEKGGQFSLH ILNHSIIVMK PRNC           594
```

ATR2$^{\Delta 2-77}$SpyTag:

```
MGNSKRVEPL KPLVIKPREE EIDDGRKKVT IFFGTQTGTA EGFAKALGEE AKARYEKTRF      60
KIVDLDDYAA DDDEYEEKLK KEDVAFFFLA TYGDGEPTDN AARFYKWFTE GNDRGEWLKN     120
LKYGVFGLGN RQYEHFNKVA KVVDDILVEQ GAQRLVQVGL GDDDQCIEDD FTAWREALWP     180
ELDTILREEG DTAVATPYTA AVLEYRVSIH DSEDAKFNDI NMANGNGYTV FDAQHPYKAN     240
VAVKRELHTP ESDRSCIHLE FDIAGSGLTY ETGDHVGVLC DNLSETVDEA LRLLDMSPDT     300
YFSLHAEKED GTPISSSLPP PFPPCNLRTA LTRYACLLSS PKKSALVALA AHASDPTEAE     360
RLKHLASPAG KDEYSKWVVE SQRSLLEVMA EFPSAKPPLG VFFAGVAPRL QPRFYSISSS     420
PKIAETRIHV TCALVYEKMP TGRIHKGVCS TWMKNAVPYE KSENCSSAPI FVRQSNFKLP     480
SDSKVPIIMI GPGTGLAPFR GFLQERLALV ESGVELGPSV LFFGCRNRRM DFIYEEELQR     540
FVESGALAEL SVAFSREGPT KEYVQHKMMD KASDIWNMIS QGAYLYVCGD AKGMARDVHR     600
SLHTIAQEQG SMDSTKAEGF VKNLQTSGRY LRDVWAHIVM VDAYKPTK                 648
```

**Supplementary Fig. 5. Protein identification of SpySystem-linked CYP73A5-ATR2 heterodimer (II).** The target band indicated in Supplementary Fig. 3 lane 2 was cut off from the gel, cleaved by trypsin, and then subjected to LC-MS/MS analysis. The matched peptides in the sample were highlighted, covering 80.08% of the full-length amino acid sequence of heterodimer (II).

CYP73A5$^{\Delta 2-28}$SpyCatcher:

```
MKKLKLPPGP IPIPIFGNWL QVGDDLNHRN LVDYAKKFGD LFLLRMGQRN LVVVSSPDLT      60
KEVLLTQGVE FGSRTRNVVF DIFTGKGQDM VFTVYGEHWR KMRRIMTVPF FTNKVVQQNR     120
EGWEFEAASV VEDVKKNPDS ATKGIVLRKR LQLMMYNNMF RIMFDRRFES EDDPLFLRLK     180
ALNGERSRLA QSFEYNYGDF IPILRPFLRG YLKICQDVKD RRIALFKKYF VDERKQIASS     240
KPTGSEGLKC AIDHILEAEQ KGEINEDNVL YIVENINVAA IETTLWSIEW GIAELVNHPE     300
IQSKLRNELD TVLGPGVQVT EPDLHKLPYL QAVVKETLRL RMAIPLLVPH MNLHDAKLAG     360
YDIPAESKIL VNAWWLANNP NSWKKPEEFR PERFFEEESH VEANGNDFRY VPFGVGRRSC     420
PGIILALPIL GITIGRMVQN FELLPPPGQS KVDTSEKGGQ FSLHILNHSI IVMKPRNCGA     480
MVDTLSGLSS EQGQSGDMTI EEDSATHIKF SKRDEDGKEL AGATMELRDS SGKTISTWIS     540
DGQVKDFYLY PGKYTFVETA APDGYEVATA ITFTVNEQGQ VTVNGKATKG DAHI           594
```

ATR2$^{\Delta 2-77}$SpyTag:

```
MGNSKRVEPL KPLVIKPREE EIDDGRKKVT IFFGTQTGTA EGFAKALGEE AKARYEKTRF      60
KIVDLDDYAA DDDEYEEKLK KEDVAFFFLA TYGDGEPTDN AARFYKWFTE GNDRGEWLKN     120
LKYGVFGLGN RQYEHFNKVA KVVDDILVEQ GAQRLVQVGL GDDDQCIEDD FTAWREALWP     180
ELDTILREEG DTAVATPYTA AVLEYRVSIH DSEDAKFNDI NMANGNGYTV FDAQHPYKAN     240
VAVKRELHTP ESDRSCIHLE FDIAGSGLTY ETGDHVGVLC DNLSETVDEA LRLLDMSPDT     300
YFSLHAEKED GTPISSSLPP PFPPCNLRTA LTRYACLLSS PKKSALVALA AHASDPTEAE     360
RLKHLASPAG KDEYSKWVVE SQRSLLEVMA EFPSAKPPLG VFFAGVAPRL QPRFYSISSS     420
PKIAETRIHV TCALVYEKMP TGRIHKGVCS TWMKNAVPYE KSENCSSAPI FVRQSNFKLP     480
SDSKVPIIMI GPGTGLAPFR GFLQERLALV ESGVELGPSV LFFGCRNRRM DFIYEEELQR     540
FVESGALAEL SVAFSREGPT KEYVQHKMMD KASDIWNMIS QGAYLYVCGD AKGMARDVHR     600
SLHTIAQEQG SMDSTKAEGF VKNLQTSGRY LRDVWAHIVM VDAYKPTK                 648
```

**Supplementary Fig. 6. Protein identification of SpySystem-linked CYP73A5-ATR2 heterodimer (III).** The target band indicated in Supplementary Fig. 3 lane 3 was cut off from the gel, cleaved by trypsin, and then subjected to LC-MS/MS analysis. The matched peptides in the sample were highlighted, covering 85.65% of the full-length amino acid sequence of heterodimer (III).

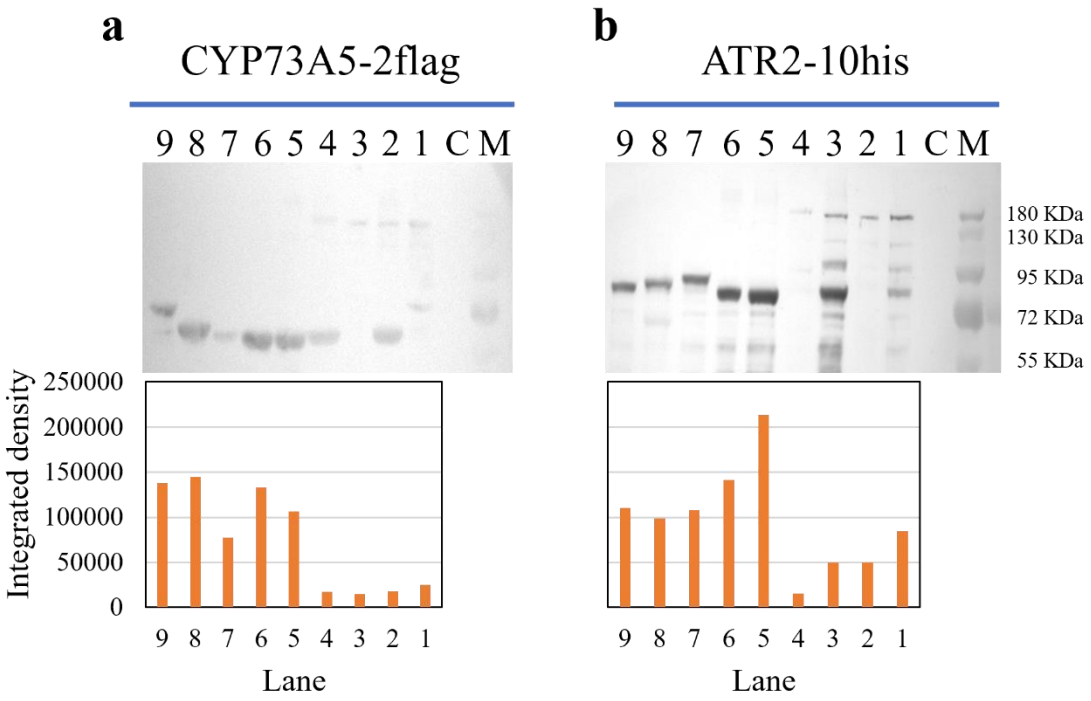
**SpyCatcherCYP73A5$^{\Delta 2\text{-}28}$:**

```
MGAMVDTLSG LSSEQGQSGD MTIEEDSATH IKFSKRDEDG KELAGATMEL RDSSGKTIST    60
WISDGQVKDF YLYPGKYTFV ETAAPDGYEV ATAITFTVNE QGQVTVNGKA TKGDAHIKKL   120
KLPPGPIPIP IFGNWLQVGD DLNHRNLVDY AKKFGDLFLL RMGQRNLVVV SSPDLTKEVL   180
LTQGVEFGSR TRNVVFDIFT GKGQDMVFTV YGEHWRKMRR IMTVPFFTNK VVQQNREGWE   240
FEAASVVEDV KKNPDSATKG IVLRKRLQLM MYNNMFRIMF DRRFESEDDP LFLRLKALNG   300
ERSRLAQSFE YNYGDFIPIL RPFLRGYLKI CQDVKDRRIA LFKKYFVDER KQIASSKPTG   360
SEGLKCAIDH ILEAEQKGEI NEDNVLYIVE NINVAAIETT LWSIEWGIAE LVNHPEIQSK   420
LRNELDTVLG PGVQVTEPDL HKLPYLQAVV KETLRLRMAI PLLVPHMNLH DAKLAGYDIP   480
AESKILVNAW WLANNPNSWK KPEEFRPERF FEEESHVEAN GNDFRYVPFG VGRRSCPGII   540
LALPILGITI GRMVQNFELL PPPGQSKVDT SEKGGQFSLH ILNHSIIVMK PRNC         594
```

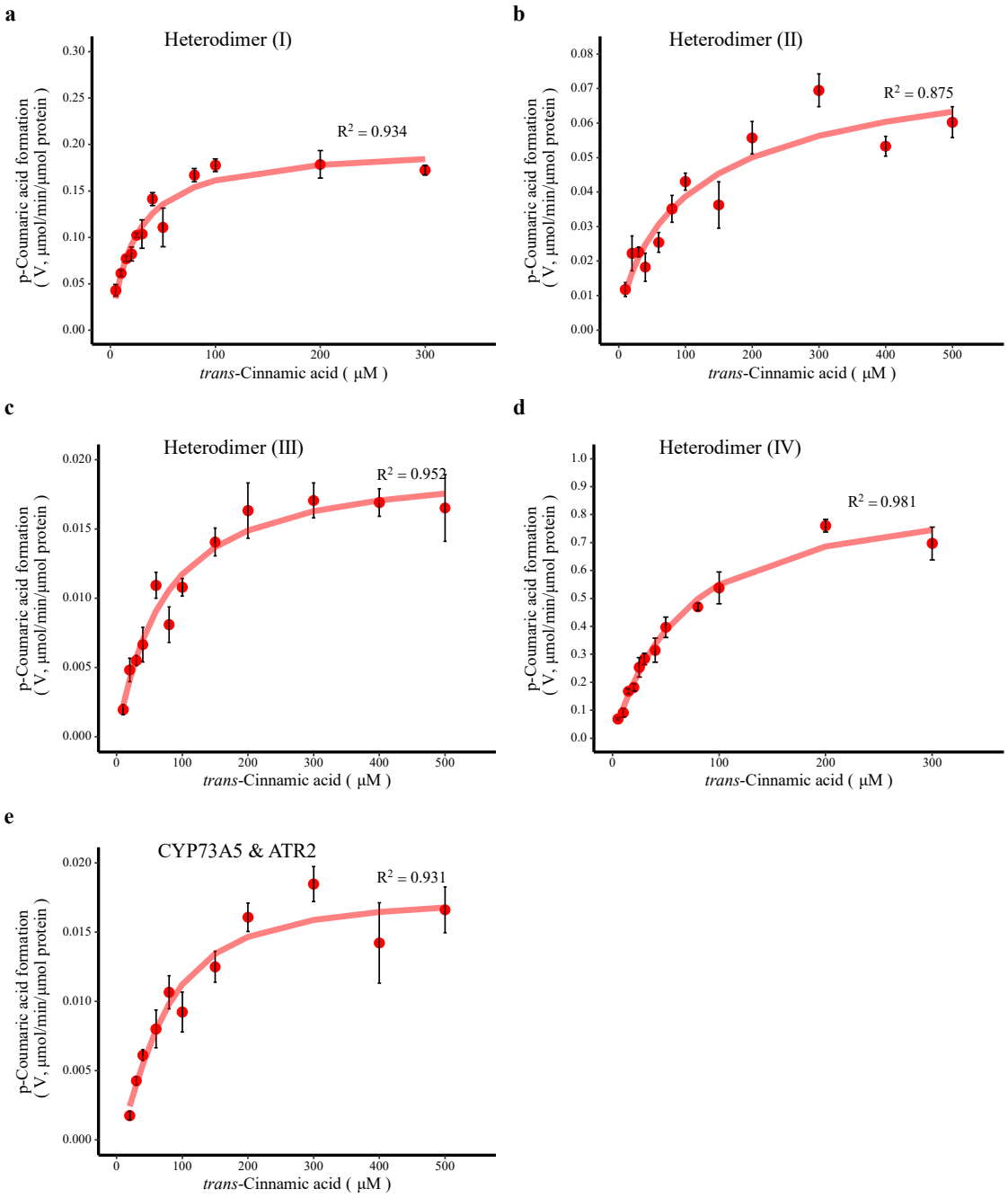
**SpyTagATR2$^{\Delta 2\text{-}77}$:**

```
MAHIVMVDAY KPTKGNSKRV EPLKPLVIKP REEEIDDGRK KVTIFFGTQT GTAEGFAKAL    60
GEEAKARYEK TRFKIVDLDD YAADDDEYEE KLKKEDVAFF FLATYGDGEP TDNAARFYKW   120
FTEGNDRGEW LKNLKYGVFG LGNRQYEHFN KVAKVVDDIL VEQGAQRLVQ VGLGDDDQCI   180
EDDFTAWREA LWPELDTILR EEGDTAVATP YTAAVLEYRV SIHDSEDAKF NDINMANGNG   240
YTVFDAQHPY KANVAVKREL HTPESDRSCI HLEFDIAGSG LTYETGDHVG VLCDNLSETV   300
DEALRLLDMS PDTYFSLHAE KEDGTPISSS LPPPFPPCNL RTALTRYACL LSSPKKSALV   360
ALAAHASDPT EAERLKHLAS PAGKDEYSKW VVESQRSLLE VMAEFPSAKP PLGVFFAGVA   420
PRLQPRFYSI SSSPKIAETR IHVTCALVYE KMPTGRIHKG VCSTWMKNAV PYEKSENCSS   480
APIFVRQSNF KLPSDSKVPI IMIGPGTGLA PFRGFLQERL ALVESGVELG PSVLFFGCRN   540
RRMDFIYEEE LQRFVESGAL AELSVAFSRE GPTKEYVQHK MMDKASDIWN MISQGAYLYV   600
CGDAKGMARD VHRSLHTIAQ EQGSMDSTKA EGFVKNLQTS GRYLRDVW               648
```

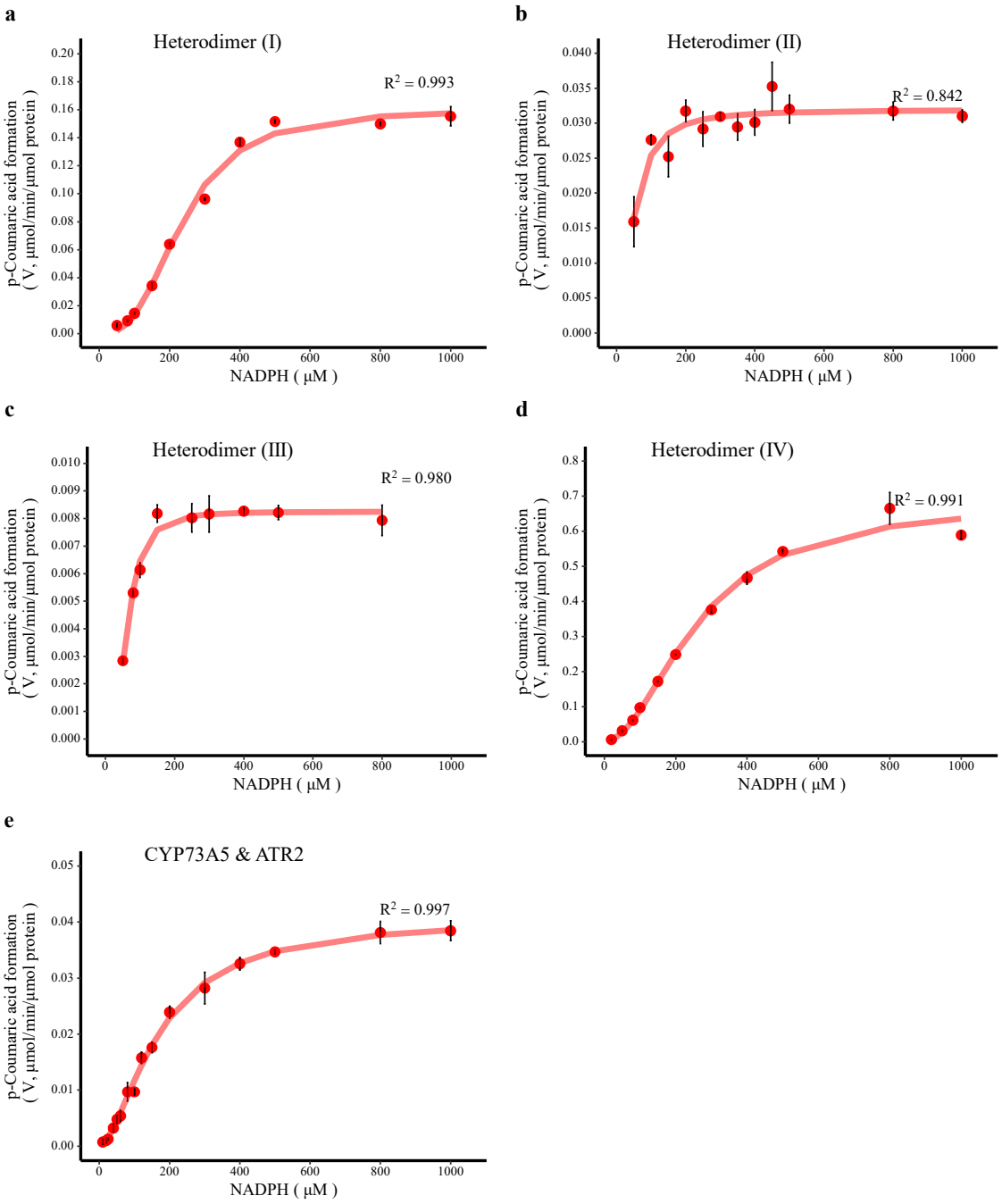
**Supplementary Fig. 7. Protein identification of SpySystem-linked CYP73A5-ATR2 heterodimer (IV).** The target band indicated in Supplementary Fig. 3 lane 4 was cut off from the gel, cleaved by trypsin, and then subjected to LC-MS/MS analysis. The matched peptides in the sample were highlighted, covering 78.31% of the full-length amino acid sequence of heterodimer (IV).



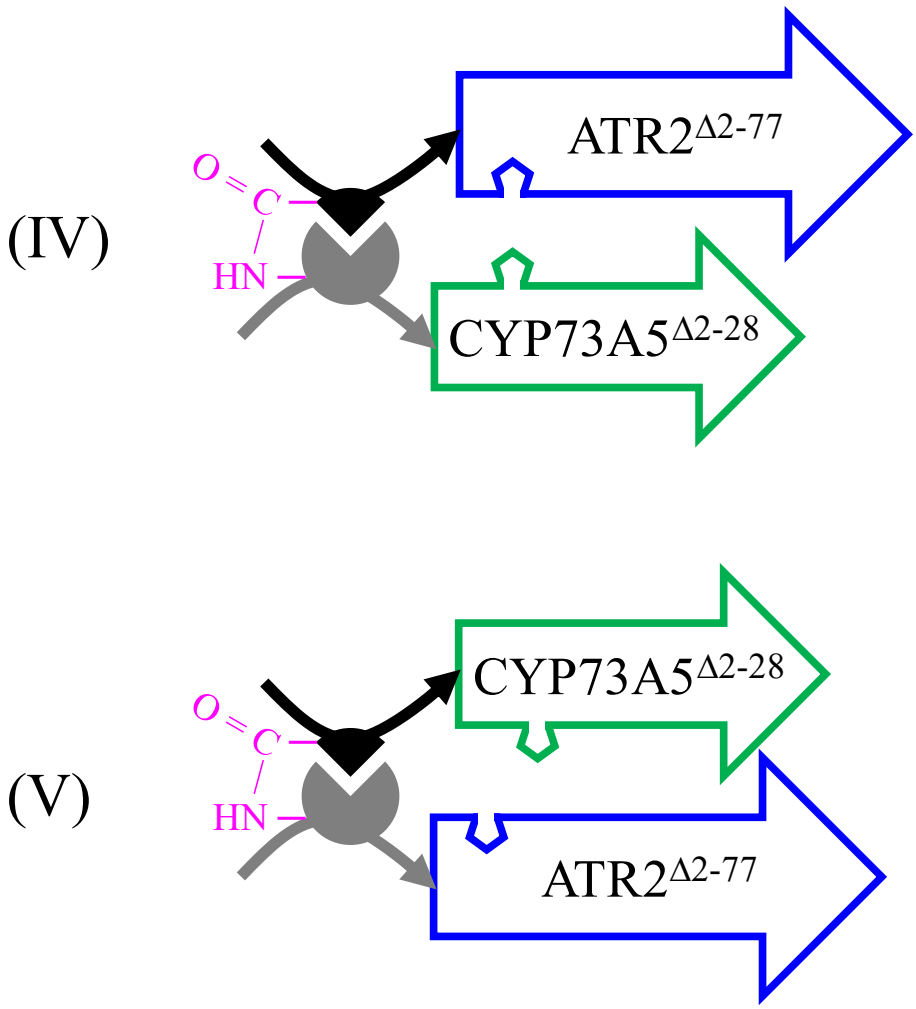

**Supplementary Fig. 8. Quantitative detection of CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$.** (**a**) CYP73A5$^{\Delta 2\text{-}28}$; (**b**) ATR2$^{\Delta 2\text{-}77}$. For Western blotting analysis, CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$ in various forms were respectively linked with two repeated Flag tags and histidine decapeptide at the C-terminus. Each lane in panel (a) was loaded with extracts of approximately 0.39 mg cells (wet weight), while that in panel (b) was 0.46 mg. Lane M, protein marker with corresponding molecular weight on the right side; Lane C, sample of the induced JIB1911 cells with the empty vector; Lane 1, sample of the induced JIB1963 cells with SpyCatcherCYP73A5$^{\Delta 2\text{-}28}$-2flag and SpyTagATR2$^{\Delta 2\text{-}77}$-10his, of which the band about 143.57 KDa is the covalent Heterodimer (IV); Lane 2, sample of the induced JIB1967 cells with SpyTagCYP73A5$^{\Delta 2\text{-}28}$-2flag and SpyCatcherATR2$^{\Delta 2\text{-}77}$-10his, of which the band about 143.57 KDa is the covalent Heterodimer (V); Lane 3, sample of the induced JIB1968 cells with SnoopCatcherCYP73A5$^{\Delta 2\text{-}28}$-2flag and SnoopTagATR2$^{\Delta 2\text{-}77}$-10his, of which the band about 143.70 KDa is the covalent Heterodimer (VI); Lane 4, sample of the induced JIB1969 cells with SnoopTagCYP73A5$^{\Delta 2\text{-}28}$-2flag and SnoopCatcherATR2$^{\Delta 2\text{-}77}$-10his, of which the band about 143.70 KDa is the covalent Heterodimer (VII); Lane 5, sample of the induced JIB1961 cells with CYP73A5$^{\Delta 2\text{-}28}$-2flag and ATR2$^{\Delta 2\text{-}77}$-10his; Lane 6, sample of the induced JIB1962 cells with 8RPCYP73A5$^{\Delta 2\text{-}28}$-2flag and 8RPATR2$^{\Delta 2\text{-}77}$-10his; Lane 7, sample of the induced JIB1965 cells with SH3ligCYP73A5$^{\Delta 2\text{-}28}$-2flag and SH3ATR2$^{\Delta 2\text{-}77}$-10his; Lane 8, sample of the induced JIB1966 cells with SH3CYP73A5$^{\Delta 2\text{-}28}$-2flag and SH3ligATR2$^{\Delta 2\text{-}77}$-10his; and Lane 9, sample of the induced JIB1964 cells with SpyCatcherCYP73A5$^{\Delta 2\text{-}28}$-2flag and SpyTag$^{mut}$ATR2$^{\Delta 2\text{-}77}$-10his. The integrated density of the target bands was measured and shown below the gels. The experiment was repeated two times with similar results. Source data are provided as a Source Data file.



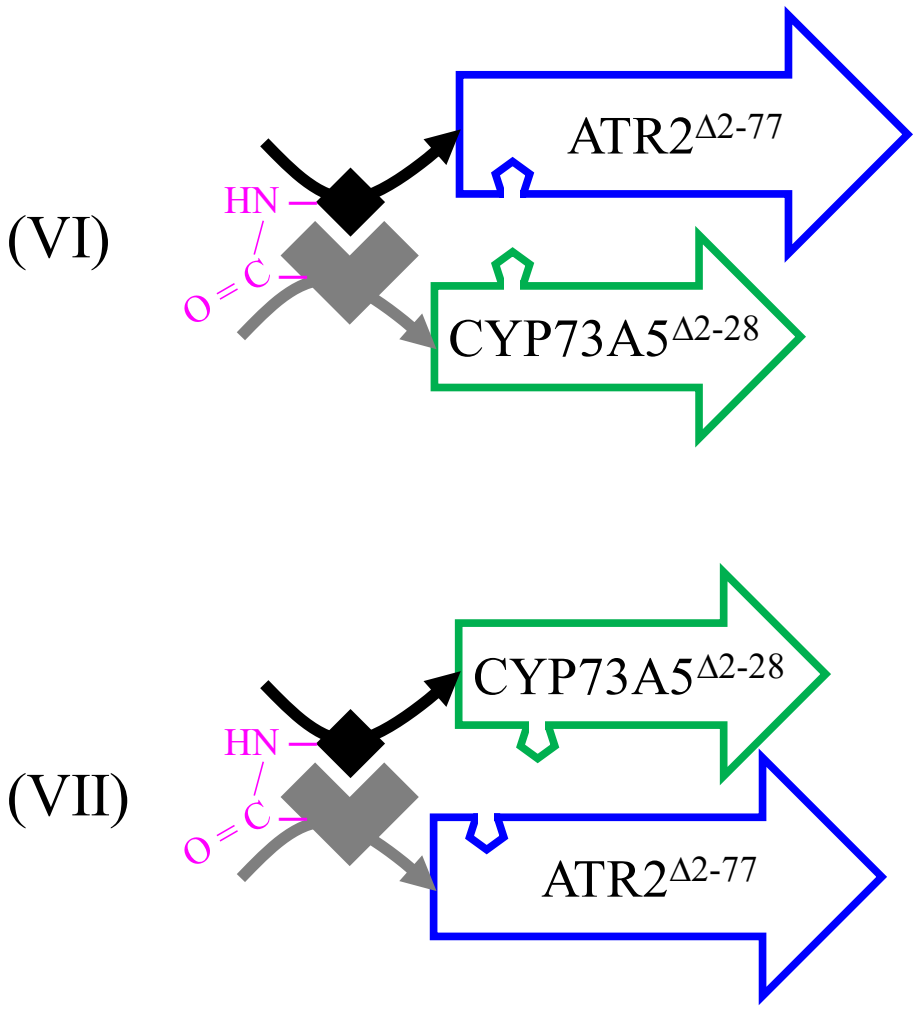

**Supplementary Fig. 9. Enzymatic kinetics of SpySystem-mediated CYP73A5-ATR2 heterodimers as well as the free-floating CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$ at increasing concentrations of the P450 substrate *trans*-cinnamic acid.** (**a**) Heterodimer (I); (**b**) Heterodimer (II); (**c**) Heterodimer (III); (**d**) Heterodimer (IV); (**e**) the free-floating individuals. The Hill's equation was used to fit the formation rate of *p*-coumaric acid to the concentrations of *trans*-cinnamic acid, and the R square was indicated besides the fitted curve. Data are shown as mean ± Standard Error (SE) (n = 3 independent experiments). Source data are provided as a Source Data file.



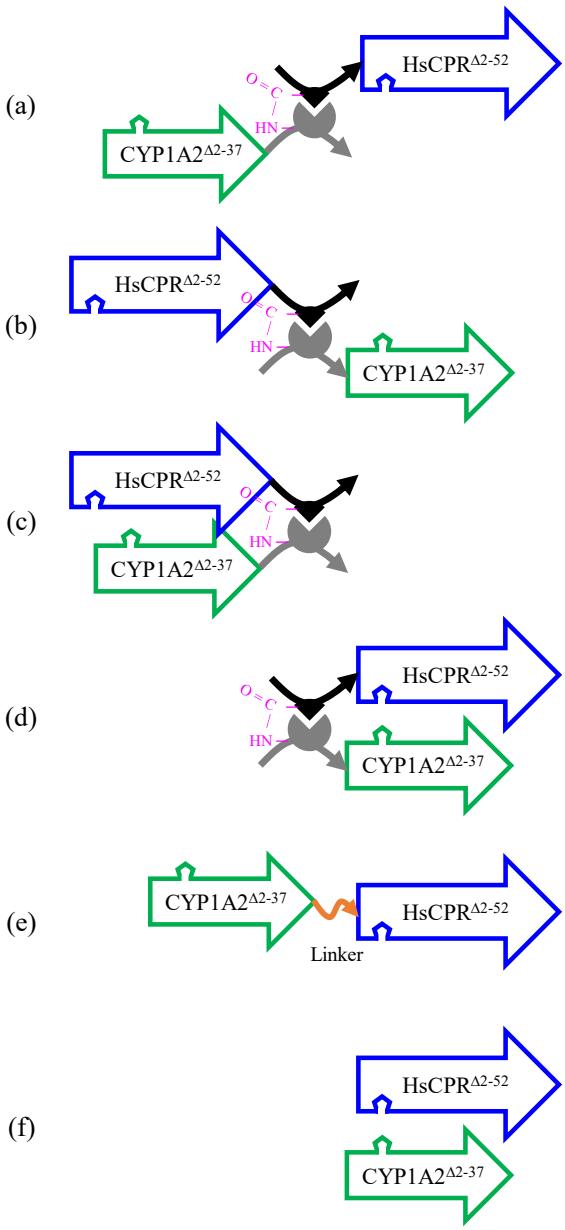

**Supplementary Fig. 10. Enzymatic kinetics of SpySystem-mediated CYP73A5-ATR2 heterodimers as well as the free-floating CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$ at increasing concentrations of the electron donor NADPH.** (**a**) Heterodimer (I); (**b**) Heterodimer (II); (**c**) Heterodimer (III); (**d**) Heterodimer (IV); (**e**) the free-floating individuals. The Hill's equation was used to fit the formation rate of *p*-coumaric acid to the concentrations of NADPH, and the R square was indicated besides the fitted curve. Data are shown as mean ± SE (n = 3 independent experiments). Source data are provided as a Source Data file.




**Supplementary Fig. 11. Configurational modulation of covalent N-termini-bridged CYP73A5-ATR2 heterodimer.** Via swapping the N-terminal peptide appendixes SpyCatcher (gray) and SpyTag (black) between CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$ within covalent heterodimer (IV), SpyCatcherCYP73A5$^{\Delta 2\text{-}28}$-SpyTagATR2$^{\Delta 2\text{-}77}$, the alternative covalent heterodimer (V), SpyTagCYP73A5$^{\Delta 2\text{-}28}$-SpyCatcherATR2$^{\Delta 2\text{-}77}$, was then created. The magenta amide bond indicates the intermolecular isopeptide bond, forming spontaneously between the side chains of aspartate in SpyTag peptide and lysine in SpyCatcher peptide at the posttranslational level [1]. The arrow indicates a polypeptide from the N-terminus to the C-terminus.




**Supplementary Fig. 12. Alternative covalent N-termini-bridged self-assembly of CYP73A5 and ATR2.** SnoopTag peptide and SnoopCatcher peptide of the lysine-asparagine peptide ligation system SnoopSystem[2] were reconstructed to the N-terminus of CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$, respectively, to mediate the formation of the N-termini-bridged heterodimer. By swapping the N-terminal appendixes SnoopCatcher (gray) and SnoopTag (black) between CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$, two covalent N-termini-bridged heterodimers, SnoopCatcherCYP73A5$^{\Delta 2\text{-}28}$-SnoopTagATR2$^{\Delta 2\text{-}77}$ (VI) and SnoopTagCYP73A5$^{\Delta 2\text{-}28}$-SnoopCatcherATR2$^{\Delta 2\text{-}77}$ (VII), were created. The magenta amide bond indicates the intermolecular isopeptide bond forming spontaneously between the side chains of lysine in SnoopTag peptide and asparagine in SnoopCatcher peptide post-translationally. The arrow indicates a polypeptide from the N-terminus to the C-terminus.




**Supplementary Fig. 13. Spatial organization of human CYP1A2 and CPR for drug bioproduction.** (**a**) SpyCatcher (gray) of the peptide ligation system SpySystem was fused to the C-terminus of CYP1A2$^{\Delta 2-37}$ while SpyTag (black) to the N-terminus of HsCPR$^{\Delta 2-52}$. (**b**) SpyCatcher (gray) was fused to the N-terminus of CYP1A2$^{\Delta 2-37}$ while SpyTag (black) to the C-terminus of HsCPR$^{\Delta 2-52}$. (**c**) SpyCatcher (gray) and SpyTag (black) were fused to the C-termini of CYP1A2$^{\Delta 2-37}$ and HsCPR$^{\Delta 2-52}$, respectively. (**d**) SpyCatcher (gray) and SpyTag (black) were fused to the N-termini of CYP1A2$^{\Delta 2-37}$ and HsCPR$^{\Delta 2-52}$, respectively. (**e**) CYP1A2$^{\Delta 2-37}$ was expressed in a tandem pattern at the N-terminus of HsCPR$^{\Delta 2-52}$ with a flexible peptide linker as an inserted hinge, indicated by the orange curve with a single arrow. (**f**) CYP1A2$^{\Delta 2-37}$ and HsCPR$^{\Delta 2-52}$ were co-expressed individually in free-floating pattern. The superscripts indicate that the N-terminal anchors of CYP1A2 (amino acid residues 2 to 37) and HsCPR (amino acid residues 2 to 52) were truncated. The magenta amide bonds indicate the intermolecular isopeptide bond forming spontaneously between the side chains of aspartate in SpyTag peptide and lysine in SpyCatcher peptide in SpySystem post-translationally. The arrow indicates a polypeptide from the N-terminus to the C-terminus.

**Supplementary Table 1. The biomass-specific productivity of *p*-coumaric acid for the strains harboring CYP73A5$^{\Delta 2\text{-}28}$ and ATR2$^{\Delta 2\text{-}77}$ in different expression pattern.** Source data are provided as a Source Data file.

| | Expression pattern | Biomass-specific Productivity (μg/g DW/h) | n |
|---|---|---|---|
| Tandem fused chimera | (i) CYP73A5$^{\Delta 2\text{-}28}$-L-ATR2$^{\Delta 2\text{-}77}$ | 86 ± 12 | 9 |
| | (ii) ATR2$^{\Delta 2\text{-}77}$-L-CYP73A5$^{\Delta 2\text{-}28}$ | 43 ± 3 | 9 |
| Free-floating individuals | MCYP73A5$^{\Delta 2\text{-}28}$ & MATR2$^{\Delta 2\text{-}77}$ | 172 ± 10 | 15 |
| | 8RPCYP73A5$^{\Delta 2\text{-}28}$ & 8RPATR2$^{\Delta 2\text{-}77}$ | 252 ± 21 | 15 |
| Heterodimer | (I) CYP73A5$^{\Delta 2\text{-}28}$SpyCatcher-SpyTagATR2$^{\Delta 2\text{-}77}$ | 192 ± 16 | 11 |
| | (II) SpyCatcherCYP73A5$^{\Delta 2\text{-}28}$-ATR2$^{\Delta 2\text{-}77}$SpyTag | 52 ± 10 | 10 |
| | (III) CYP73A5$^{\Delta 2\text{-}28}$SpyCatcher-ATR2$^{\Delta 2\text{-}77}$SpyTag | 85 ± 13 | 12 |
| | (IV) SpyCatcherCYP73A5$^{\Delta 2\text{-}28}$-SpyTagATR2$^{\Delta 2\text{-}77}$ | 563 ± 17 | 12 |
| | (V) SpyTagCYP73A5$^{\Delta 2\text{-}28}$-SpyCatcherATR2$^{\Delta 2\text{-}77}$ | 286 ± 28 | 9 |
| | (VI) SnoopCatherCYP73A5$^{\Delta 2\text{-}28}$-SnoopTagATR2$^{\Delta 2\text{-}77}$ | 465 ± 42 | 14 |
| | (VII) SnoopTagCYP73A5$^{\Delta 2\text{-}28}$-SnoopCatherATR2$^{\Delta 2\text{-}77}$ | 557 ± 42 | 12 |
| | (VIII) SH3ligCYP73A5$^{\Delta 2\text{-}28}$/SH3ATR2$^{\Delta 2\text{-}77}$ | 612 ± 29 | 9 |
| | (IX) SH3CYP73A5$^{\Delta 2\text{-}28}$/SH3ligATR2$^{\Delta 2\text{-}77}$ | 357 ± 30 | 9 |
| | SpyCatcher$^{mut}$CYP73A5$^{\Delta 2\text{-}28}$/SpyTagATR2$^{\Delta 2\text{-}77}$ | 204 ± 13 | 8 |
| | SpyCatcherCYP73A5$^{\Delta 2\text{-}28}$/SpyTag$^{mut}$ATR2$^{\Delta 2\text{-}77}$ | 373 ± 19 | 9 |
| | SpyCatcher$^{mut}$CYP73A5$^{\Delta 2\text{-}28}$/SpyTag$^{mut}$ATR2$^{\Delta 2\text{-}77}$ | 344 ± 47 | 9 |

## Supplementary references


1. Zakeri, B. et al. Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. *Proc. Natl Acad. Sci. USA* **109**, e690–e697 (2012).
2. Veggiani, G. et al. Programmable polyproteams built using twin peptide superglues. *Proc. Natl Acad. Sci. USA* **113**, 1202–1207 (2016).