Engineering Protein Farnesyltransferase for Enzymatic Protein Labeling Applications

Jonathan K. Dozier,[†] Santoshkumar L. Khatwani,[†] James Wollack,[†] Yen-Chih Wang,[†] Claudia Schmidt-[‡] and Mark D. Distefano[†*]

[†]Department of Chemistry, University of Minnesota, 207 Pleasant St SE, Minneapolis, MN 55455

[‡]Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, 1479 Gortner Avenue, St. Paul, MN 55108

**Correspondence to*: Mark Distefano (Telephone: (+612) 624-0544; Fax: (+612) 626-7541; E-mail: diste001@umn.edu)

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Primers of PFTase Mutants

Forward Primer W102A:
5′-tgccagccgccccgcgctctgctactgg-3′
Translated 5′-3′ Frame 2
tgccagccgccccgcgctctgctactgg
A S R P A L C Y W

Reverse Primer W102A:
5′-ccagtagcagagcgcggggcggctggca-3′
Translated 3′-5′ Frame 2
tgccagccgccccgcgctctgctactgg
A S R P A L C Y W

Forward Primer Y154A:
cacctcgctcccacggctgcagctgtcaacgc
Translated 5′-3′ Frame 1
cacctcgctcccacggctgcagctgtcaacgc
H L A P T A A A V N

Reverse Primer Y154A:
gcgttgacagctgcagccgtgggagcgaggtg
Translated 3′-5′ Frame 1
cacctcgctcccacggctgcagctgtcaacgc
H L A P T A A A V N

Forward Primer Y205A:
5′-gtggatgtaagaagtgcggcctgtgctgcctcagtagc-3′
Translated 5′-3′ Frame 1
gtggatgtaagaagtgcggcctgtgctgcctcagtagc
V D V R S A A C A A S V

Reverse Primer Y205A:
5′-gctactgaggcagcacaggccgcacttcttacatccac-3′
Translated 3′-5′ Frame 1
gtggatgtaagaagtgcggcctgtgctgcctcagtagc
V D V R S A A C A A S V

LC-MS Analysis of CNTF-NBD

CNTF-CVIA, as prepared earlier (*1*), was prenylated with the NBD analogue. A reaction consisting of 50 mM Tris HCl (pH= 7.5), 50 μM $ZnCl_2$, 10 mM $MgCl_2$, 20 mM KCl, 15 mM DTT, 10 μM NBD-FPP analogue (**5**), 2 μM CNTF-CVIA and 100 nM Y205A PFTase(*2*)(*1*) was prepared. This solution was allowed to react for 4 h at 30 °C. The reaction was submitted directly for LC-MS analysis to determine CNTF prenylation. The LC method used was a gradient of 3% B to 97% B (Mobile Phase A: H_2O, 0.1% HCO_2H; Mobile Phase B: CH_3CN, 0.1% HCO_2H) in 15 min. Mass spectra (*m/z* 300-2500) were collected every 0.2 s during the chromatographic separation.

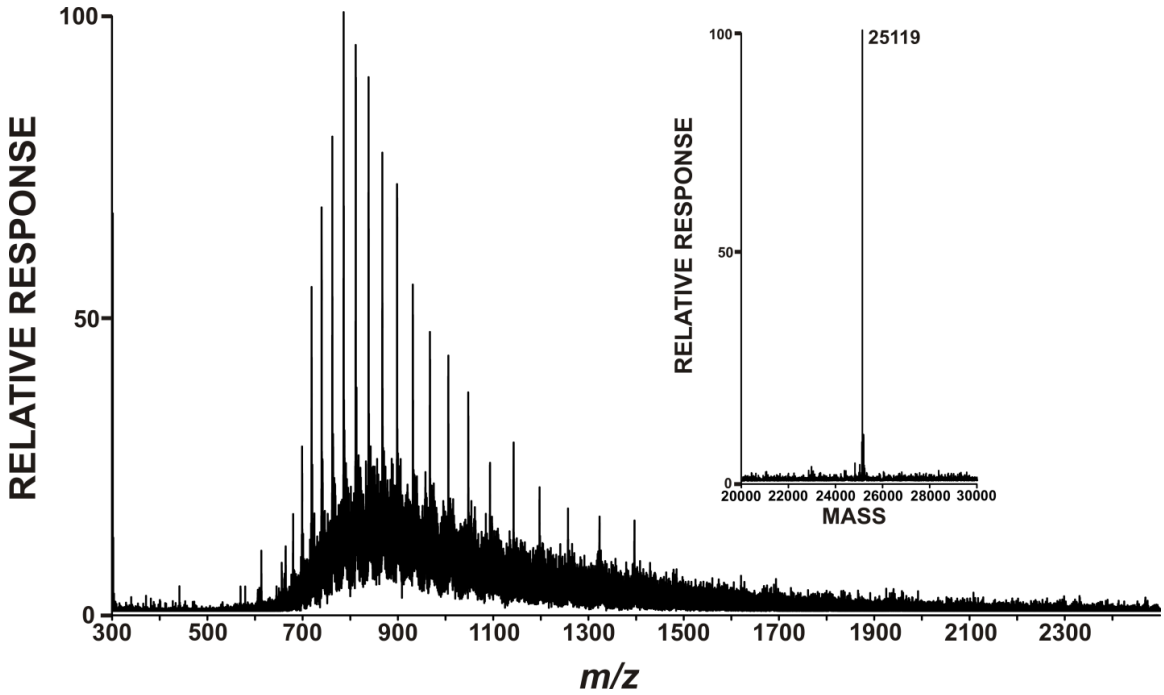

Figure S1. ESI-MS spectrum of prenylated CNTF with NBD-FPP analogue (**5**) with the deconvoluted mass spectrum shown in the insert. The calculated mass was 25,120.6 and the observed mass was 25,119 which is within the accuracy range of the mass spectrometer for a protein of this mass.

SDS-PAGE Fluorescence Analysis of CNTF-CVIA prenylation of NBD-FPP Analogue

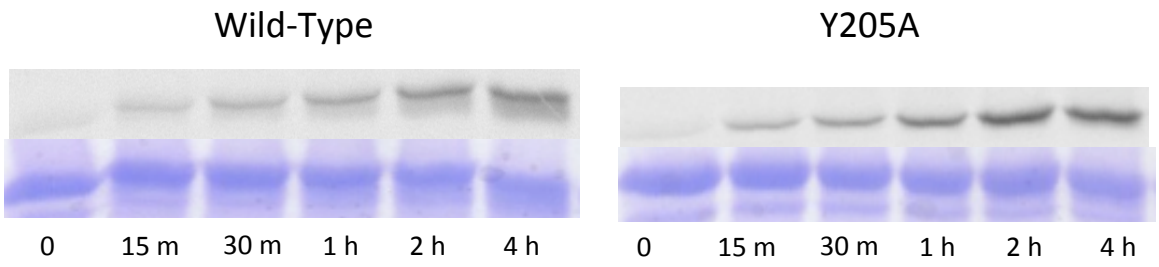

Figure S2. Gels used to determine the amount of prenylation of CNTF-CVIA with the NBD-FPP analogue (**5**). Gels were scanned for fluorescence with a Storm 840 Fluorescence scanner with 450 nm excitation wavelength and 520 nm long pass emission filter. The top gel is the fluorescent scan and the bottom gel is the total protein stain using Coomassie blue.

Stereoscopic Image of PFTase Binding Site

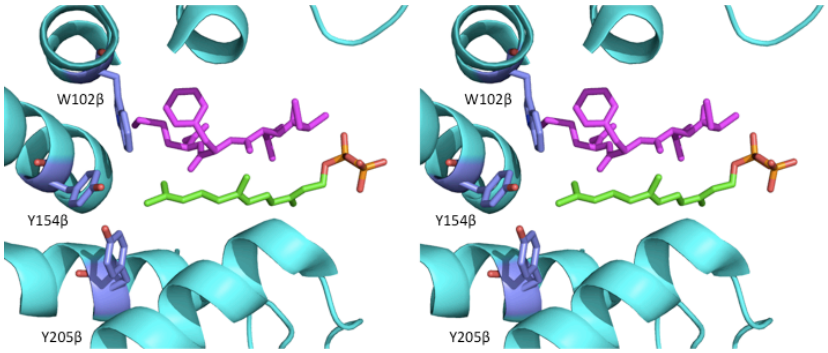

Figure S3. Stereoscopic image of Figure 2a. Protein shown as cartoon in light blue. Three key residues are shown in blue. Peptide backbone shown in pink. FPP shown in green. Oxygens on amino acid residues and FPP shown in red. Phosphorous shown as orange.

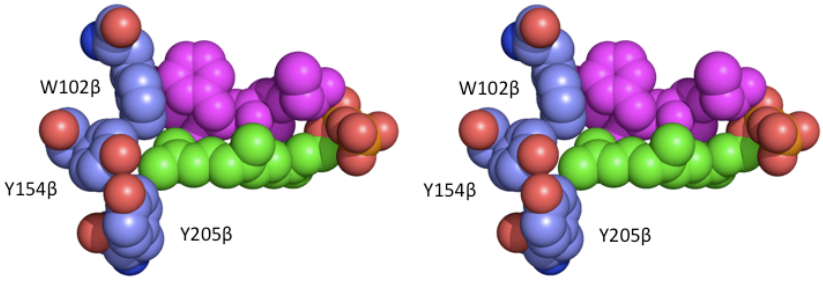

Figure S4. Stereoscopic image of Figure 2b. Space filling model of binding pocket. Three key residues are shown in blue. Peptide backbone shown in pink. FPP shown in green. Oxygens on amino acid residues and FPP shown in red. Phosphorous shown as orange.

Stereoscopic Image of Aldehyde Analogue Binding

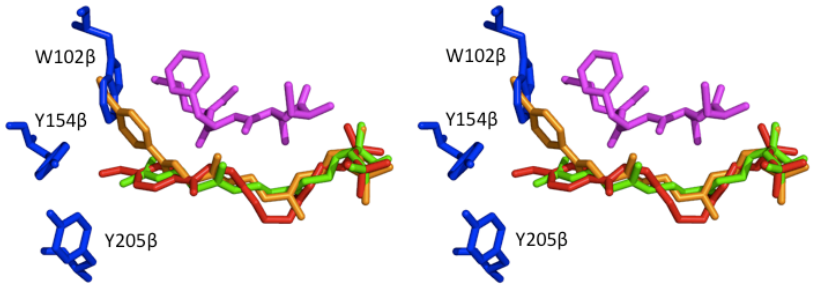

Figure S5. Stereoscopic image of Figure 5a. Stick representation of binding of FPP and aldehyde containing analogues bound in both wild-type and W102Aβ enzymes. Three key amino acids shown in blue, peptide substrate shown in pink, natural bound FPP shown in green, aldehyde analogue bound into the wild-type enzyme shown in red, aldehyde analogue bound into the W102Aβ enzyme shown in orange.

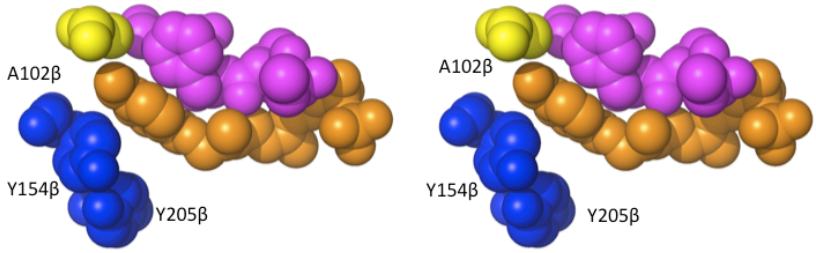

Figure S6. Stereoscopic image of Figure 5b. Space filling model of binding of aldehyde containing analogue bound in W102Aβ enzyme. Two key amino acids shown in blue, mutated tryptophan to alanine shown in yellow, peptide substrate shown in pink, aldehyde analogue bound into the W102Aβ enzyme shown in orange.

Stereoscopic Image of Coumarin Analogue Binding

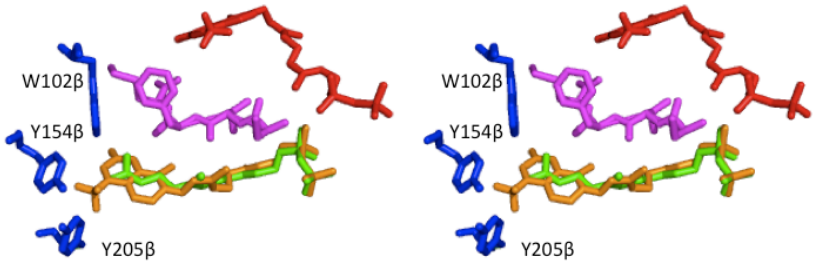

Figure S7. Stereoscopic image of Figure 6a. Stick representation of binding of FPP and coumarin containing analogue bound in both wild-type and Y205Aβ enzymes. Three key amino acids shown in blue, peptide substrate shown in pink, natural bound FPP shown in green, coumarin analogue bound into the wild-type enzyme shown in red, coumarin analogue bound into the Y205Aβ enzyme shown in orange.

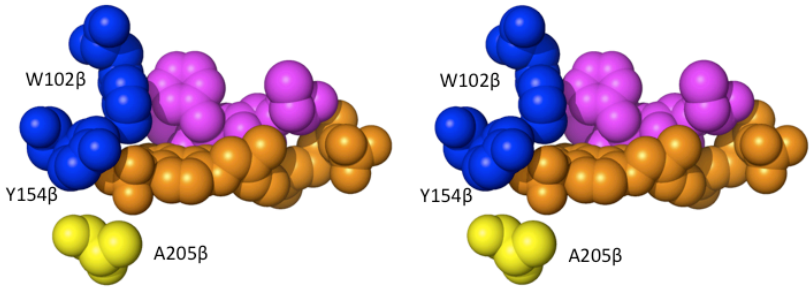

Figure S8. Stereoscopic image of Figure 6b. Space filling model of binding of aldehyde containing analogue bound in Y205Aβ enzyme. Two key amino acids shown in blue, mutated tyrosine to alanine shown in yellow, peptide substrate shown in pink, aldehyde analogue bound into the Y205Aβ enzyme shown in orange.

FRET Analysis of GFP-CVIA to GFP-CVLL

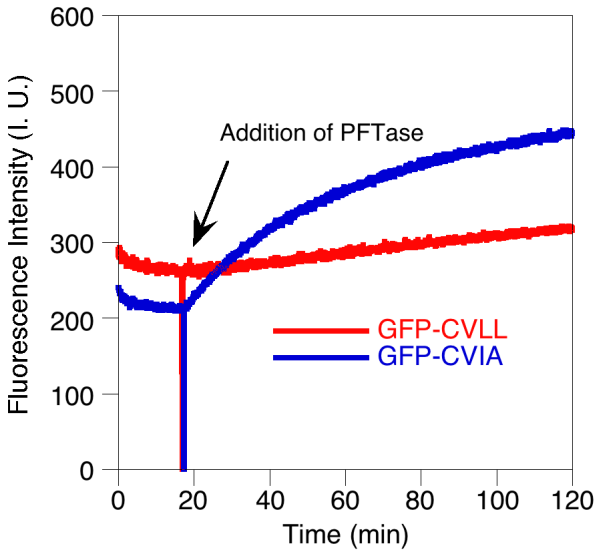

Figure S9. Continuous FRET assay comparing the changes observed using GFP-CVLL (red) and GFP-CVIA (blue) as substrates. Data shows emission intensity at 510 nm (λ_{ex} = 330 nm) after the addition of PFTase.

Mass Spectrometric Data for Coumarin Labeled GFP

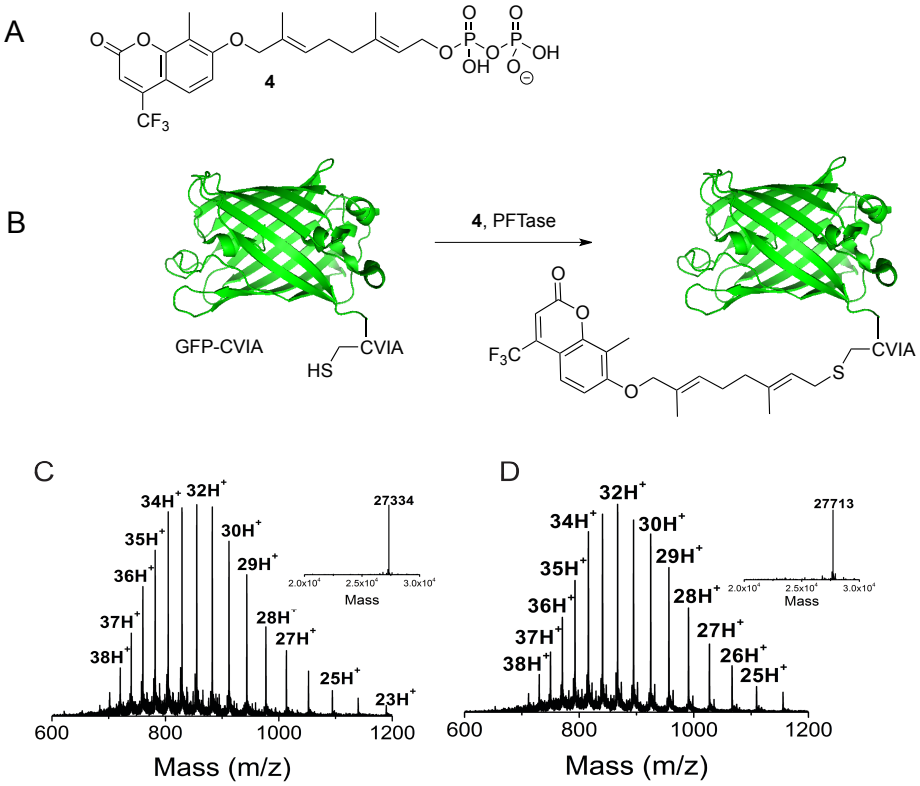

Figure S10. Labeling of GFP-CVIA with coumarin analogue: (A) Structure of coumarin-based analogue (**4**). (B) Schematic representation of labeling of a protein containing C-terminal CaaX sequence (GFP-CVIA) with **4** via enzymatic reaction to yield a fluorescently-labeled prenylated protein. ESI-MS spectra of unlabeled GFP-CVIA (C) and coumarin-labeled GFP-CVIA (D) are shown with their respective deconvoluted mass spectra shown in the insets.

Kinetic Analysis with Isoprenoid Analogues

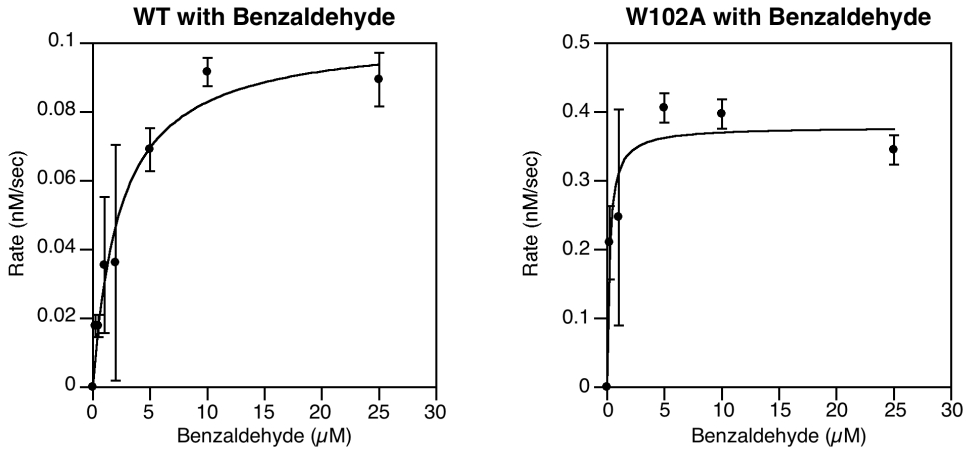

Figure S11. Rate versus substrate concentration plots for the reaction of the benzaldehyde analogue **2** with the wild-type enzyme and the W102A mutant enzyme. The kinetic parameters K_M and V_{max} were obtained by non-linear least squares direct fitting of this data to the Michaelis-Menton equation.

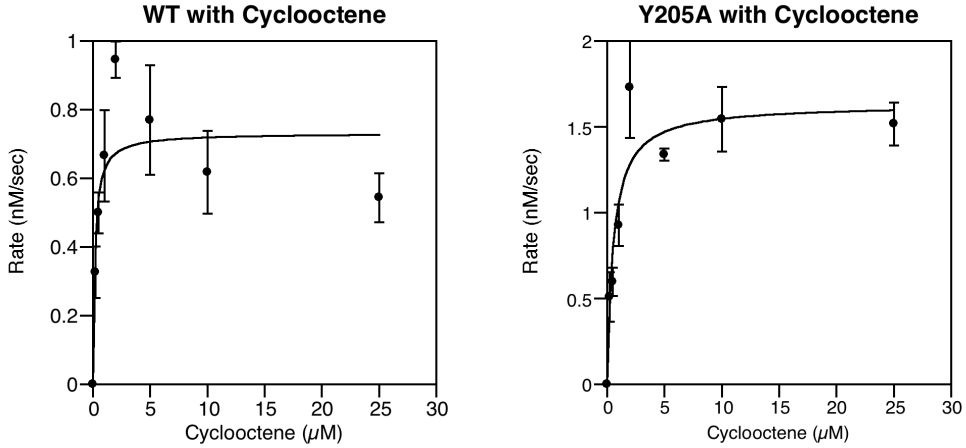

Figure S12. Rate versus substrate concentration plots for the reaction of the trans-cyclooctene analogue **3** with the wild-type enzyme and the Y205A mutant enzyme. The kinetic parameters K_M and V_{max} were obtained by non-linear least squares direct fitting of this data to the Michaelis-Menton equation.

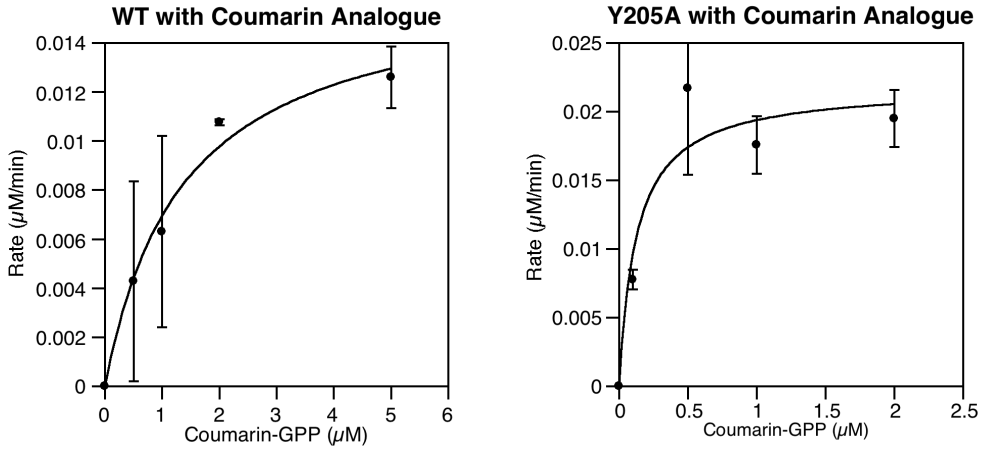

Figure S13. Rate versus substrate concentration plots for the reaction of the coumarin analogue **4** with the wild-type enzyme and the Y205A mutant enzyme. The kinetic parameters K_M and V_{max} were obtained by non-linear least squares direct fitting of this data to the Michaelis-Menton equation.

References

(1) Rashidian, M., Kumarapperuma, S. C., Gabrielse, K., Fegan, A., Wagner, C. R., and Distefano, M. D. (2013) Simultaneous Dual Protein Labeling Using a Triorthogonal Reagent. *J. Am. Chem. Soc. 135*, 16388–16396.

(2) Dozier, J. K., and Distefano, M. D. (2012) An enzyme-coupled continuous fluorescence assay for farnesyl diphosphate synthases. *Anal. Biochem. 421*, 158-163.