Supporting Information

for

Exploiting the Substrate Tolerance of Farnesyltransferase for Site-Selective
Protein Derivatization

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The first synthetic approach for azo-substituted FPP involved the introduction of the azide by direct transformation of the hydroxyl group present in compound 1 in order to obtain compound 20. Attempts using Mitsunobu reaction or mesylate displacement by sodium azide, triflyl azide [TfN₃][42] or diphenylphosphoryl azide [(PhO)₂PON₃][43] in various conditions led either to no reaction or to an inseparable mixture of compounds 20 and 21 (Scheme S1 A), resulting from both Sₙ2 and Sₙ2' reaction pathways.[11;12] By Using a different synthetic route that implied chain elongation, the azide moiety could be effectively and regiospecifically introduced to the molecule by successive reaction of alcohol 1 with chloroacetyl chloride and sodium azide (Scheme S1 B). However, the azidoacetate moiety present in compound 24 did not survive the conditions for the pyrophosphorylation and the cation exchange: only pyrophosphate 25, resulting from the ester hydrolysis, was finally obtained.
Scheme S1. A) Products of isothermal rearrangement of the azide in the first attempt for the synthesis of azide functionalized FPP. B) Second attempt resulted in the ester hydrolysis of 24.
a CICOCH₂Cl, Et₃N, DMAP, CH₂Cl₂, 0 °C, 86%. b NaN₃, DMF, RT, 94%. c PPTS, EtOH, 60 °C, quant. d (a) NCS, DMS, CH₂Cl₂, -40 to 0 °C; (b) (NBu₄)₃HP₂O₇, CH₃CN, RT; (c) cation exchange.

Figure S1. Titration of 160 nM NBD-FPP with FTase yielded a $K_d$ of 5 nM.
Figure S2. HPLC runs coupled to ESI-MS of the CFP-CAAX prenylation mixtures. Retention times: 11.85 min (A without prenylation), 12.35 min (B FPP), 12.20 min (C HOM-GPP), 11.90 min (D AAA-GPP) and 12.08 min (E APO-GPP).
Figure S3. ESI-MS spectra of proteins eluted as monomeric prenylated CFP-CAAX in analytical gel filtration chromatography. A CFP-CAAX-HOM-G (fraction 17 mL), B CFP-CAAX-AAA-G (fraction 17 mL) and C CFP-CAAX-APO-G (fraction 16 mL). All spectra showed a minor peak of [M-131]$^+$ that reflected the removal of the N-terminal translation initiator Met by methionine aminopeptidase. In addition, the spectrum of A showed a typical fragmentation peak of [M-80]$^+$ corresponding to the loss of the hexadienyl group during ionization.

Figure S4. A SDS-PAGE analysis of the prenylation mixture with the respective analogues. Lane 1: standard marker, lane 2: CFP-CAAX-HOM-G, lane 3: CFP-CAAX-AAA-G, lane 4: CFP-CAAX-APO-G. B Spectrofluorometric titration of Ypt7-CAAX-NBD-G with MRS6. Conditions: 25 mM Hepes, pH 7.2, 40 mM NaCl, 2 mM MgCl₂, 2 mM DTE, 10 µM GDP, 0.2% N-octylglycosyl-β-thiopyranoside. The concentration of the complex was 100 nM. The excitation/emission wavelengths were fixed at 487/530 nm. Data were fitted to a quadratic equation as implemented in the program Graphit 5.1 (Erithacus software) leading to a $K_d$ of 275.4 ± 19.8 nM.
Figure S5. Ligation of CFP-CAAX-HOM-G with 6-maleimidohexanoic acid. A), B) ESI-MS spectra of CFP-CAAX-HOM-G reacted at pH 6.0 and 7.2, respectively. Unligated protein: 29974 Da, expected ligated protein: 30185 Da. C), D) ESI-MS spectra of control reactions of CFP-CAAX-F incubated with reagent at pH 6.0 and 7.2, respectively. Unligated protein: 29945 Da, expected ligated protein: 30156 Da. All spectra showed a minor peak of \([M-131]^+\) that reflected the removal of the N-terminal translation initiator Met by methionine aminopeptidase. In addition, the spectrum of A and B showed a typical fragmentation peak of \([M-80]^+\) corresponding to the loss of the hexadienyl group during ionization.