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Supporting Information

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Development and Biological Evaluation of Acyl Protein Thioesterase 1 (APT 1) Inhibitors

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Determination of the IC_{50} value

The described compounds were incubated with APT1 and the S-palmitoylated lipopentapeptide fragment Ac-Met-Ser-Cys(Pal)-Lys-Cys(Far)OMe corresponding to the C-terminus **1** of the H-Ras protein. The released palmitate, which correlates with the enzyme activity was

measured according to the ADIFAB assay-protocol described by Richieri et al.[10] For each inhibitor concentration the measurement of the fluorescence emission was determined six times.

Rat APT1 was isolated as described[7] and stored at –78°C in aqueous solution containing 50 mM HEPES pH = 8.0, 2 mM $MgCl_2$, 1 mM EDTA und 7.5 mM CHAPS. ADIFAB (Molecular Probes) was stored under similar conditions in 50 mM Tris-HCl pH = 8.0, 1 mM EDTA and 0.05% NaN_3. The assay buffer contained 20 mM HEPES pH = 7.4, 150 mM NaCl, 5 mM KCl, and 1 mM Na_2HPO_4. The concentrations for substrate, APT1 and ADIFAB were 1 µM, 1.6 pM and 100 nM respectively.

Stereoselectivity of APT1

Depalmitoylation of D-configured Ras peptide **17** and the corresponding enantiomer incorporating L-amino acids by APT1 was determined with the ADIFAB assay.[10] For each substrate the measurement was repeated four times (Figure 2).

Spectral shift of the geranyl-mant labelled N-Ras lipoprotein

Analogous to the experiments employing the H-Ras based C-terminal peptide **18** the substitution of the natural farnesyl isoprenoid by a geranyl/N-Methylanthranyl-group should allow for the spectroscopical monitoring of palmitoylation in a corresponding N-Ras lipoprotein (**16**). Fluorescence emission spectra (370 – 480 nm) of the N-Ras protein **16** alone or after incubation with APT1 and / or palmitoyl coenzyme A were measured upon excitation at 360 nm (Fig. 3). Incubation with APT1 alone had no influence on fluorescence emission quantum yield or the wavelength of the maximum (Fig. 3A). Addition of palmitoyl CoA to the

fluorescent palmitoylatable Ras-protein resulted in a small increase in quantum yield (Fig. 3B). In contrast, presence of both, APT1 and palmitoyl CoA, resulted in a hypsochromic shift of the fluorescence emission maximum from 427 nm to 421 nm (Fig. 3), indicating a decrease in the polarity of the fluorophore environment. As shown for the H-Ras based C-terminal peptide **18**, where a palmitoylated reference compound was also investigated, this shift can be utilized as a signal for N-Ras S-palmitoylation. The relatively small hypsochromic shift, 6 nm compared to 30 nm in the case of the H-Ras peptide, is probably due to the bigger distance between the palmitoylatable cysteine and the fluorescent farnesyl analogue in the case of the C-terminal N-Ras sequence (Figure 3).

Palmitoylation of N-ras employing [^{3}H]-labelled palmitic acid

To a reaction mixture with 100 nM N-Ras and 10 µM [9, 10-^{3}H]palmitic acid 100 nM APT1 was added giving a final volume of 100 µl. The buffer was as described above (Figure 2).

5 µl of the reaction mixture were taken before, 2 min and 20 min after addition of APT1 and submitted to SDS-PAGE (stacking gel 5%, separating gel 15%). Due to the low concentrations used 2 µl of a 10 µM N-Ras stock solution was added to 5 µl 2x gel buffer to which then the 5 µl of the reaction mixture was added. After running the gel for an hour at a constant voltage of 120 V the gel was stained with Coomassie Brilliant Blue for an hour. The gel was then destained for an hour with 10% acetic acid, 5% methanol in distilled water. The corresponding N-Ras bands were then cut out and incubated in 3 ml scintillation fluid

(Ready Protein$^+$, Beckman-Coulter) over night and then submitted to scintillation counting (LS 6500, Beckman-Coulter).

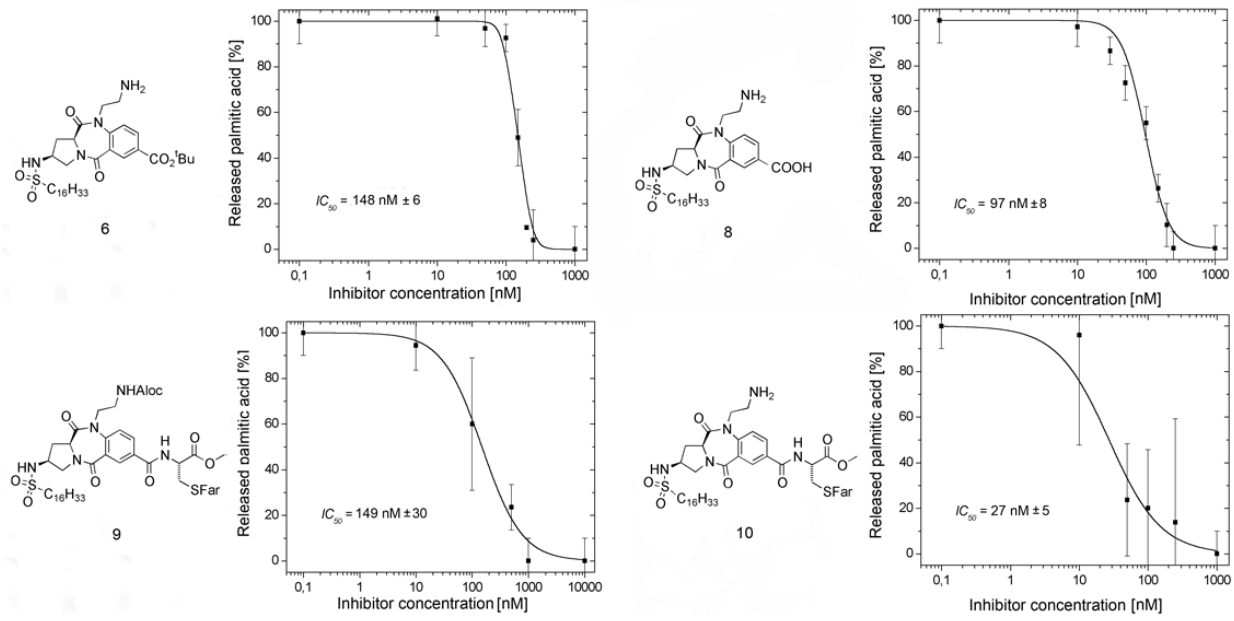

Figure 1 Inhibition of APT1 by inhibitors **6**, **8**, **9** and **10**; determination of the IC_{50} value.

The described compounds were incubated with APT1 and the S-palmitoylated lipopentapeptide fragment Ac-Met-Ser-Cys(Pal)-Lys-Cys(Far)OMe corresponding to the C-terminus **1** of the H-Ras protein. The released palmitate, which correlates with the enzyme activity was measured according to the ADIFAB assay-protocol described by Richieri et al.[10] For each inhibitor concentration the measurement of the fluorescence emission was determined six times.

Rat APT1 was isolated as described[7] and stored at –78°C in aqueous solution containing 50 mM HEPES pH = 8.0, 2 mM $MgCl_2$, 1 mM EDTA und 7.5 mM CHAPS. ADIFAB (Molecular Probes) was stored under similar

conditions in 50 mM Tris-HCl pH = 8.0, 1 mM EDTA and 0.05% NaN_3. The assay buffer contained 20 mM HEPES pH = 7.4, 150 mM NaCl, 5 mM KCl, and 1 mM Na_2HPO_4. The concentrations for substrate, APT1 and ADIFAB were 1 µM, 1.6 pM and 100 nM respectively.

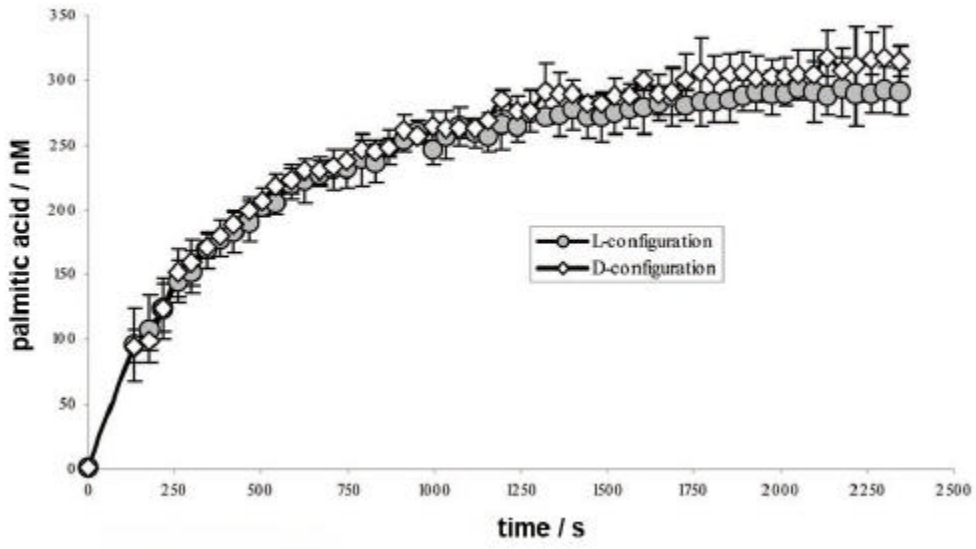

Figure 2 Depalmitoylation of D-configured Ras peptide **17** and the corresponding enantiomer incorporating L-amino acids by APT1. The enzymatic activity is displayed as liberated fatty acid (in nM) over time (in sec) determined with the ADIFAB assay.[10] Grey circles: C-terminus of N-Ras in L-configuration; white squares: C-terminus of N-Ras in D-configuration. For each substrate the measurement was repeated four times. Vertical bars: standard deviation. The mean standard deviation for the substrates in L- and D-configuration was 15 nM and 13 nM respectively.

APT1 was stored at –20 °C in aqueous solution containing 50 mM Tris-HCl pH 8,0; 150 mM NaCl; 5 mM DTE and 1 mM EDTA. ADIFAB was stored under similar conditions in 50 mM Tris-HCl, pH 8.0; 1 mM EDTA and 0.05 % (w/v) NaN_3.

The assay buffer contained 20 mM HEPESNa, pH 7.4; 150 mM NaCl; 5 mM KCl; 1 mM Na_2HPO_4. All buffers and solutions were degassed by sonification and kept under argon until use. Assay-concentrations for substrate, APT1 and ADIFAB were 1 µM, 1 µM and 100 nM respectively.

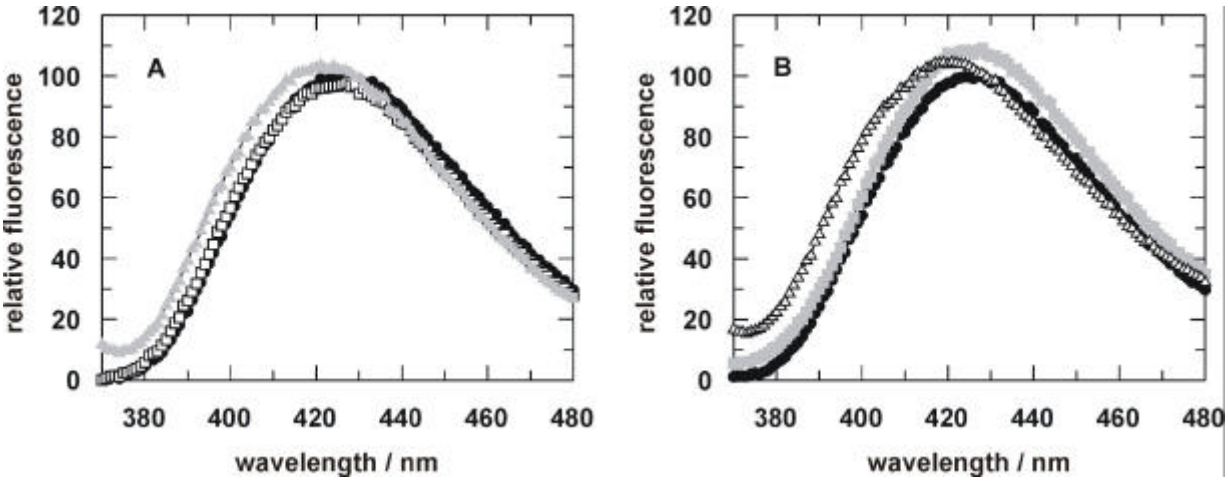

Figure 3 Change in fluorescence emission spectra (370 – 480 nm, excitation at 360 nm) of N-Ras lipoprotein **16** (500 nM) incubated with APT1 (5 µM) and / or palmitoyl coenzyme A (50 µM). Measurements were performed at 20 °C in 20 mM Hepes, pH 7.4, 150 mM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, spectra were corrected for Raman scattering (substraction of buffer spectra) and normalized for the fluorescence emission maximum of the N-Ras protein **16** alone (black circles). Detection of spectral changes upon addition of

either APT1 or palmitoyl CoA was performed after an incubation period of 30 minutes each. In **A** first APT1 was added to N-Ras (open squares), followed by incubation with palmitoyl CoA (grey triangles). In **B** the N-Ras protein **16** was first incubated with palmitoyl coenzyme A (grey squares). Here, addition of APT1 was done as a second step (open triangles).