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Supporting Information

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Supporting Information

for

Mapping the Isoprenoid Binding Pocket of PDEd by a Semisynthetic, Photoactivatable N-Ras Lipoprotein

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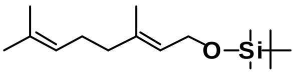
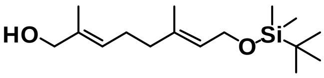
Procedures for the synthesis of benzophenone labeled cysteinederivate 8.

Geranyl-*t*-butyldimethylsilylether (2)



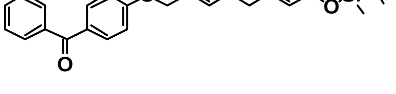
The cysteine-building-block (CBB) was synthesized as follows. To a solution of geraniol (8.76 mL, 0.05 mol, 1 equiv) and DIPEA (17.39 mL, 0.10 mol, 2 equiv) in dry DCM (50 mL) at 0°C, a solution of *t*-butyldimethylsilylchloride (7.53 g, 0.05 mol, 1 equiv) in dry DCM (10 mL) is added at 0°C. After 4h stirring the solution was washed five times with HCl-solution (0.2M) and afterwards once with sat. NaCl solution (50 mL). The organic layer was dried with $MgSO_4$. After filtration the solvent was evaporated and the product was obtained as yellow oil. Yield: 13.2g (48.6 mmol, 97%); GC-MS (DB_100_S): t_R = 4.14 min; *m/z* 268 (6 %), 211 (23 %), 135 (30 %), 75 (100 %); ^{1}H NMR (400 MHz, $CDCl_3$): d= 0.07 (s, 6H, 11-H), 0.90 (s, 9H), 1.60 (s, 3H), 1.62 (s, 3H), 1.68 (s, 3H), 1.98-2.12 (m, 4H), 4.19 (d, ^3J = 6.24 Hz, 2H), 5.10 (t, ^3J = 1.46 Hz, 1H), 5.30 ppm (t, ^3J = 1.96 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): d= -4.6 (CH_3), 16.6 (CH_3), 18.0 (C_q,), 18.7 (CH_3), 26.0 (CH_3), 26.3 (CH_3), 26.7 (CH_2), 39.88 (CH_2), 60.7 (CH_2), 124.4 (CH), 124.7 (CH), 131.8 (C_q), 137.1 ppm (C_q).

8-Hydroxy-geranyl-*t*-butyldimethylsilylether (3)



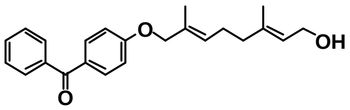
To a mixture of seleniumdioxide (0.291 g, 2.62 mmol, 0.1 equiv), salicylic acid (0.362 g, 2.62 mmol, 0.1 equiv) and DCM (150 mL) *tert*-butylhydroperoxid (13.08 mL, 104.48 mmol, 4eq, 80% in (*t*BuO)$_2$-H_2O) was added slowly and under stirring. After 30 min at 0°C geranyl-*t*-butyldimethylsilylether (7 g, 26.12 mmol, 1 equiv) was added. After 5h stirring at room temperature the solvent was removed in vacuo (water temperature <36°C), and the residue was coevaporated twice with toluene (100 mL). The residue was diluted with diethyl ether (50 mL) and washed twice with sat. $NaHCO_3$ (10 mL). The organic layer was dried with $MgSO_4$ and after filtration the solvent was removed. The residue was purified by column chromatography with cyclohexane/ ethyl acetate (5:1). The product was obtained as a yellow oil. R_f=0.30 (cyclohexane / ethyl acetate 5:1); Yield: 1.36 g (4.78 mmol, 18.5 %); GC-MS (DB_100_S): t_R = 4.90 min; *m/z* 283 (1 %), 135 (28 %), 105 (27 %), 93 (30 %), 75 (100 %); ^{1}H NMR (400 MHz, $CDCl_3$): d= 0.06 (s, 6H), 0.90 (s, 9H), 1.62 (s, 3H), 1.65 (s, 3H), 2.04 (t, ^3J = 4.8 Hz, 2H), 2.14 (s, ^3J = 7.5 Hz, 2H), 3.98 (s, 2H), 4.18 (d*d, ^3J = 6.28 Hz, 2H), 5.30 (m, 1H), 5.38 ppm (m, 1H)

8-(4-Benzoylphenyloxy)-geranyl-*t*-butyldimethylsilylether (4)



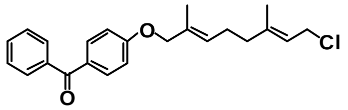
8-Hydroxy-geranyl-*t*-butyldimethylsilylether (4 g, 14.08 mmol, 1equiv) was dissolved in THF (30 mL). Triphenylphosphine (5.54 g, 21.31 mmol, 1.5 equiv) and *p*-hydroxybenzophenone (3.35 g, 16.90 mmol, 1.2 equiv) were added and finally diisopropyl azodicarboxylate (DIAD; 4.18 mL, 21.13 mmol, 1.5 equiv) was dropped slowly into the solution under ice-cooling. After 4h at room temperature ether (150 mL) and water (50 mL) were added to obtain two phases. The organic layer was washed twice with 30 mL brine and dried with $MgSO_4$. After removing the solvent in vacuo the product was obtained by chromatography (cyclohexane / ethyl acetate (10:1)), R_f = 0.32 (cyclohexane/ ethyl acetaete 10:1); Yield: 3.17 g (6.83 mmol, 48.8 %); GC-MS (DB_100_S): t_R = 10.61 min; *m/z* 407 (40 %), 333 (7 %), 249 (11 %), 207 (16 %), 105 (100 %) 75 (50 %); ^{1}H NMR (400 MHz, $CDCl_3$): d= 0.07 (s, 6H), 0.90 (s, 9H), 1.64 (s, 3H), 1.75 (s, 3H), 2.07 (t, ^3J = 7.6 Hz, 2H), 2.21 (t, ^3J = 7.6 Hz, 2H), 4.19 (d, ^3J = 6.44 Hz 2H), 4.46 (s, 2H), 5.32 (t, ^3J = 6.94 Hz, 1H), 5.56 (t, ^3J = 7.52 Hz, 1H), 4.46 (s, 2H), 6.69 (d, ^3J = 8.8 Hz, 2H), 7.47 (m, 2H), 7.55 (m, 1H), 7.75 (m, 2H), 7.81 ppm (m, 2H)

1.4 8-(4-Benzoylphenyloxy)-geraniol (5)



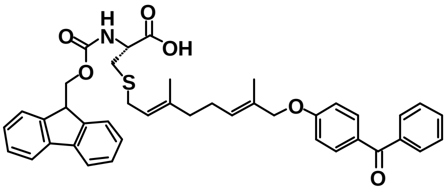
The TBDMS-ether 4 (1.63 g, 3.51 mmol, 1 equiv) was dissolved in dry THF(20 mL), and the solution was cooled to 0°C, followed by addition of TBAF (1M sol. in THF) (4.21 mL, 4.21 mmol, 1.2 equiv) under stirring. After the colour has changed from yellow to orange the mixture was stirred for one additional hour, diluted with ethyl acetate (50 mL) und washed twice with brine (20 mL). The combined organic layers were dried with $MgSO_4$. After evaporation of the solvent, column chromatography with cyclohexane / ethylacetate (1:1) yielded the product as a white, amophous solid. R_f = 0.55 (cyclohexane / ethylacetate 1:1); Yield: 1.18 g (3.37 mmol, 96 %); ^{1}H NMR (400 MHz, $CDCl_3$): d= 1.69 (s, 3H), 1.75 (s, 3H), 2.12 (t, ^3J = 7.52 Hz, 2H), 2.22 (t, ^3J = 7.52 Hz, 2H), 4.14 (d, ^3J = 6.84 Hz, 2H), 4.47 (s, 2H), 5.41 (t, ^3J = 6.74 Hz, 1H), 5.55 (t, ^3J = 7.02 Hz, 1H), 6.96 (t, ^3J = 9 Hz, 2H), 7.47 (m, 2H), 7.55 (m, 1H), 7.75 (m, 2H), 7.80 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): d= 14.5 (CH_3), 16.8 (CH_3), 26.5 (CH_2), 39.5 (CH_2), 60.0 CH_2), 74.7 (CH_2), 105.0 (CH), 124.5 (CH), 128.8 (CH), 129.5 (CH), 130.3 (CH), 131.1 (CH), 132.5 (C_q), 133.1 (C_q), 139.5 (C_q), 163.2 (C_q), 196.2 ppm (C_q)

8-(4-Benzoylphenyloxy)-geranylchloride (6)



To a solution of N-chlorsuccinimide (0.96 g, 7.22 mmol, 1.1 equiv) in DCM at -45°C, dimethylsulfide (0.97 mL, 13.12 mmol, 2 equiv) was added. After warming up to 0°C for 5 min the mixture was cooled to -40°C, and a solution of 8-(4-benzoylphenyloxy)-geraniol (2.30 g, 6.65 mmol, 1 equiv) in DCM (10 mL) was added dropwise. The resulting mixture was allowed to warm up to 0°C over 1h and stirred at 0°C for a one further hour. The mixture was diluted with cyclohexane, washed twice with ice-cold brine, dried over $MgSO_4$, and concentrated in vacuo. Because of instability the product was not purified, but used immediately in the synthesis of 8. R_f = 0.86 (cyclohexane/ ethyl acetate 1:1); NMR of the crude product:: ^{1}H NMR (400 MHz, $CDCl_3$): d= 1.60 (s, 3H), 1.74 (s, 3H), 2.12 (t, ^{3}J = 7.52 Hz, 2H), 2.22 (t, ^{3}J = 7.52 Hz, 2H), 4.08 (d, ^{3}J = 8 Hz, 2H), 4.47 (s, 2H), 5.45 (t, ^{3}J = 8.1 Hz, 1H), 5.54 (t, ^{3}J = 7.52 Hz, 1H), 6.96 (d, ^{3}J = 8.76 Hz, 2H), 7.47 (m, 2H), 7.55 (m, 1H), 7.76 (m, 2H), 7.81 ppm (m, 2H)

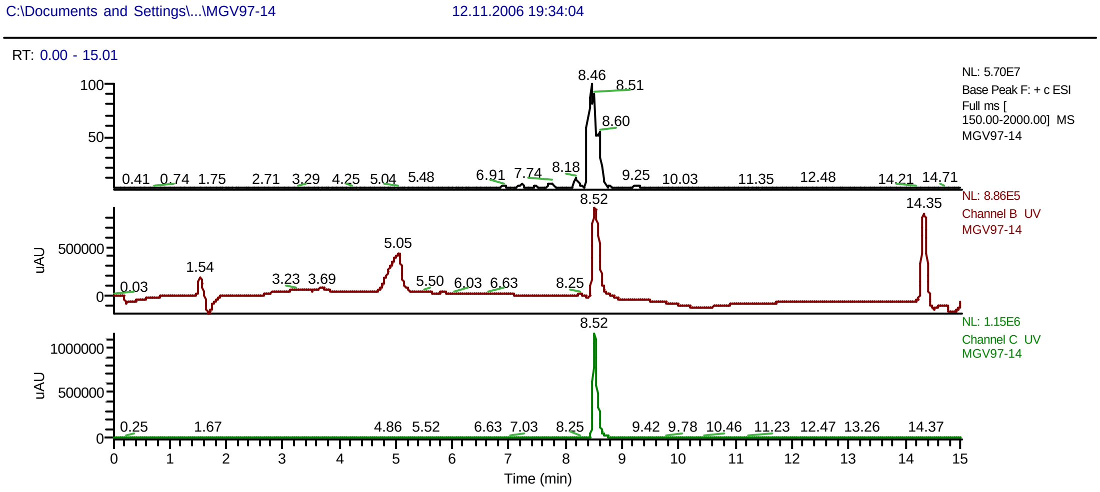
N-Fmoc-S-[8-(4-Benzoylphenyloxy)-geranyl]-L-Cystein-methylester (8)

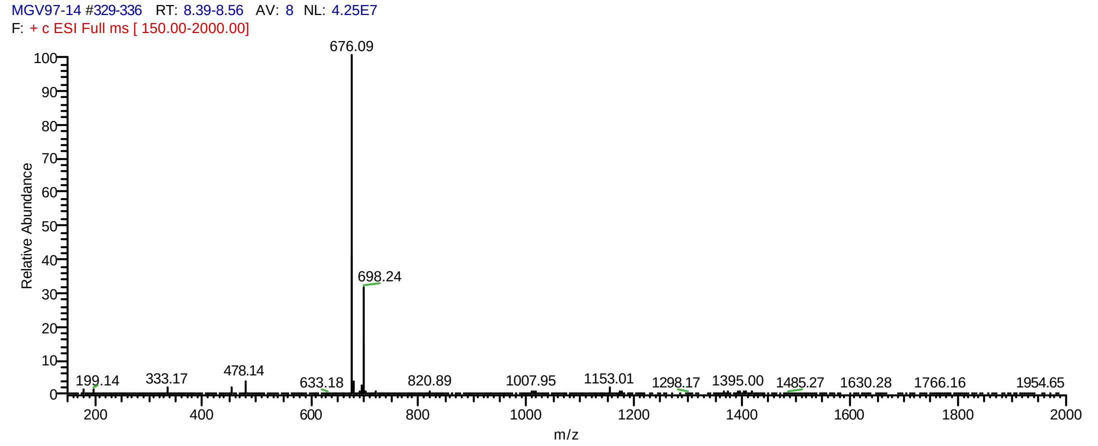


L-Cysteine-hydrochloride (1.25 g, 7.98 mmol, 1.2 equiv) was dissolved in NH_3/MeOH (7M, 18.6 mL, 100 mmol, 16 equiv) and a solution of 8-(4-benzoylphenyloxy)-geranylchloride (2.4 g, 6.56 mmol, 1 equiv) in dry MeOH (20 mL) was added dropwise at 0°C. After 3h the mixture was allowed to warm to room temperature for 2h and concentrated in vacuo to obtain a white solid.

To a solution of the crude product (6.56 mol, 1 equiv) in DCM (50 mL), triethylamine (3 mL, 21.28 mmol. 3.2 equiv) was added at room temperature. After 10 min Fmoc-OSu (7.18 g, 21.28 mol, 3.2 equiv) was added and allowed to react overnight. The product was purified by column chromatography with cyclohexane / ethyl acetate (2:1) followed by DCM / MeOH (10:1). R_f = 0.41 (DCM/MeOH 10:1); $[\alpha]_D^{20}$= 15.5 (c= 0.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): d= 1.64 (s, 3H), 1.71 (s, 3H), 2.10 (d, ^{3}J = 7.24 Hz, 2H), 2.19 (d, ^{3}J = 6.94 Hz, 2H), 2.99 (m, 2H), 3.20 (m, 2H), 4.23 (t, ^{3}J = 6.94 Hz, 1H), 4.40 (d, ^{3}J = 6,8 Hz, 2H), 4.46 (s, 2H), 4.60 (m, 1H), 5.22 (t, ^{3}J = 7.8 Hz, 1H), 5.49 (t, ^{3}J = 6.84 Hz, 1H), 6.95 (d, ^{3}J = 8.8 Hz, 2H), 7.28 (m, 2H), 7.38 (m, 2H), 7.46 (m, 2H), 7.58 (m, 3H), 7.74 (m, 4H), 7.78 ppm (m, 2H); HR-MS(FAB): for $C_{41}H_{41}N_2O_6S$ calculated: 675.2655; measured 675.2626 (M^+), 676.2715 (M^++H).

LC-MS: (C4 -column)





SDS-PAGE and silver staining

Fractionated protein samples after analytical gel filtration were pooled and identified by 15% SDS-PAGE and silver staining. Staining was performed as follows: Fixation (30´; 40 % methanol, 10 % acetic acid), incubation (10´; 30 % ethanol, 6.8 % $C_2H_3NaO_2$ x $3H_2O$, 0.02% $Na_2S_2O_3$ x 5 H_2O), purification (3x 5´ A. dest.), silver staining (10´; 0.2 % $AgNO_3$, 0.04 % formaldehyde 36 %), purification (3x 20´´ A. dest.), developing (~10´-15´; 5% Na_2CO_3, 0.02 % formaldehyde 36 %) and stop-solution (40 mM EDTA (pH 8.0), 10% glycerol).

MALDI_TOF sample preparation

Zip tips (C18 column tips, Millipore) were first activated using 50% ACN and then equilibrated using 0.1% FA (formic acid). Peptides were mixed in a 1:1 ratio (v/v) with a MALDI-matrix of saturated (10 mg mL^{-1}) α-cyano-4-hydroxycinnamic acid (CHCA) or a sinapinic acid (SA) solution mixture (50% (*v/v*) acetonitrile, 0.1% (*v/v*) trifluoroacetic acid [TFA]). Furthermore, peptides were bound to the C18 resin and desalted by washing with 0.1% FA. For analyses, 0.5 µL of the mixture was spotted on a MALDI steel target plate (100 well plate, Applied Biosystems, USA) and dried in air. To allow for the top down spotting motion with the Zip tips (Millipore, Bedford, MA, USA) matrix solution was aspirated by the bare needle. The solution was slowly dispensed through the top of the Zip tip to cause the matrix solution to elute the bound peptides onto the MALDI target and mix with them as they are spotted onto the target.

External mass calibration was performed with two calibration standards: C1 calibration mixture (Applied Biosystems; Foster City, USA) with des-Arg^1-bradykinin, (905.05; $[M+H]^+$), angiotensin I (1297.51; $[M+H]^+$), Glu^1-fibrinopeptide B (1571.61; $[M+H]^+$) and neurotensin (1673.96; $[M+H]^+$) and C2 calibration mixture (Applied Biosystems; Foster City, USA) including insulin (bovine, 5734.59 $[M+H]^+$ /2867.80 $[M+2H]^{2+}$), thioredoxin (*E. coli*, 11647.48; $[M+H]^+$ / 5837.74; $[M+2H]^{2+}$) and apomyoglobin (horse, 16952.56; $[M+H]^+$ / 8476.78; $[M+2H]^{2+}$) respectively. Both standards were mixed in a 1:1 ratio (*v/v*) with a saturated MALDI-matrix CHCA solution (50% (*v/v*) ACN, 0.1% (*v/v*) TFA). Mass analysis was performed with Voyager™ 5.1 Software Data Explorer™ (Applied Biosystems, USA) after calibration.

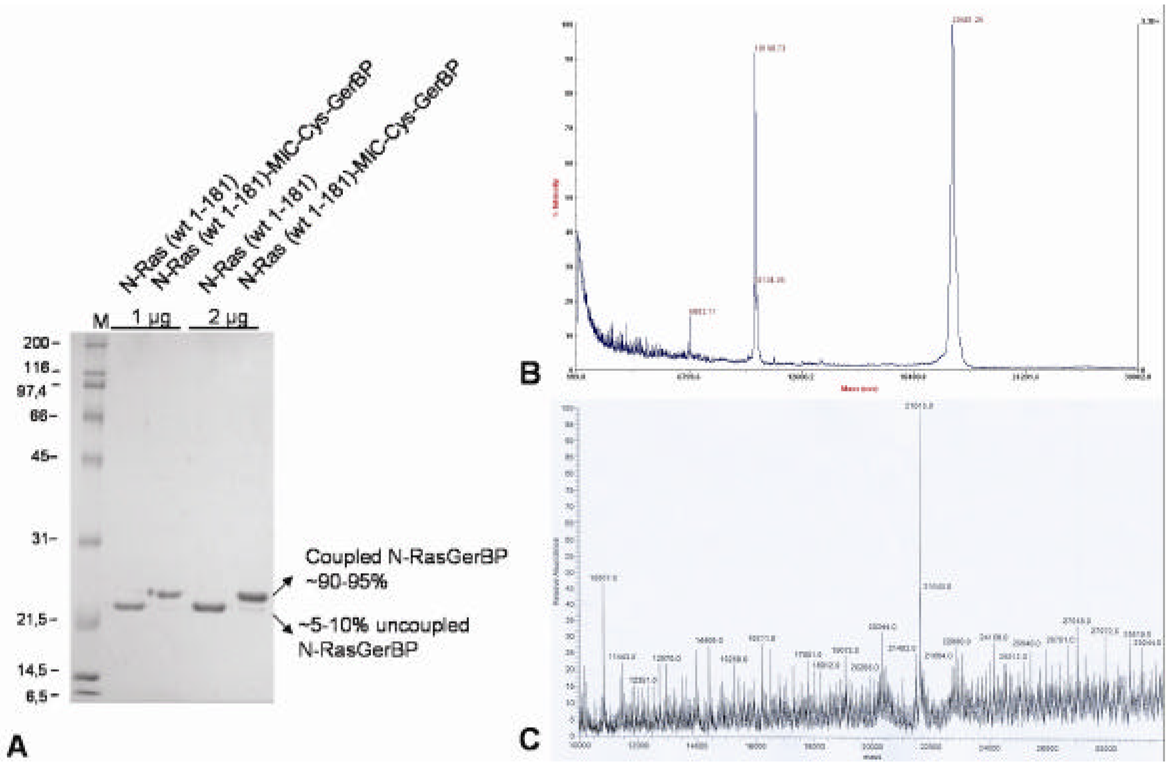
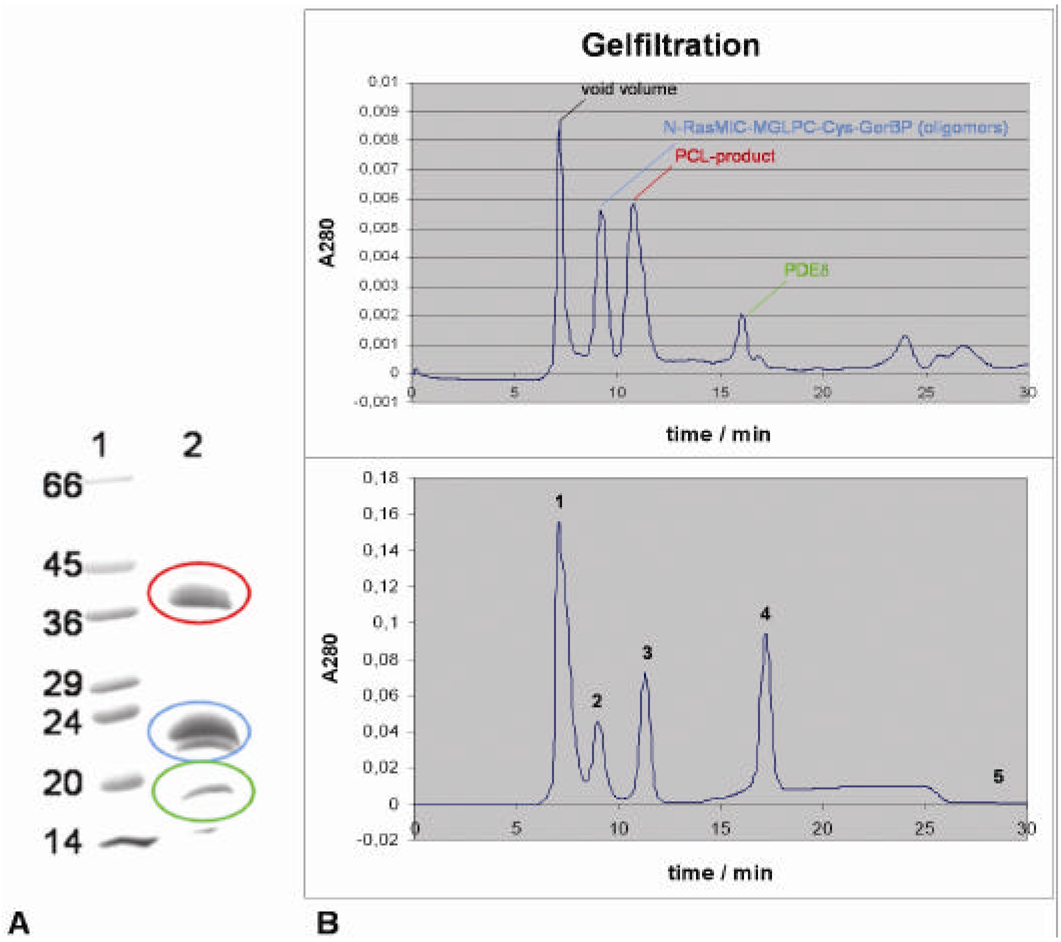


Figure S1. SDS-PAGE and MALDI-TOF MS characterization of the photoactivatable *N*-Ras lipoprotein. **A**. SDS-PAGE of *N*-Ras educt and the purified coupling educt. In lane with 2 µg lipoprotein applied a minor contamination with truncated *N*-Ras(1-181) is visible. **B**. MALDI spectrum of *N*-Ras (wt) 1-181 protein. The major signal at 20401.25 Da corresponds to a calculated mass of 20398.0 Da to the *N*-Ras (wt) 1-181 protein. The mass tolerance is Δ = 3.25 Da to the calculated mass. **C**. ESI-MS spectrum of *N*-Ras (wt) 1-181 MIC-GCMGLPC-GerBP-OMe was performed on a Finnigan LCQ spectrometer (Advantage MAX). The major signal at 21615.0 Da (calculated *N*-Ras (wt) 1-181 MIC-GCMGLPC-GerBP-OMe = 21617.534 Da; Δ = 2.534 Da) corresponds to the deprotected semisynthetic lipoprotein.



Signals (upper figure):

1= void volume

2= ~21 kDa *N*-Ras-MIC-GCMGLPC-GerBP (oligomers)

3= ~38 kDa PCL-product

4= ~17 kDa PDEd

5+6= DTE, salt

Signals (lower figure) Biosil2000-Standard (Bio-Rad)):

1= void volume + 670 kDa

2= 158 kDa

3= 44 kDa

4= 17,8 kDa

5= 1,35 kDa

Figure S2. Purification of the photo cross-linked products of *N*-Ras-GerBP and PDEd. **A**. SDS-PAGE of the reaction mixture after coupling. **B.** Gel filtration of the reaction mixture in coupling buffer with a multicomponent Waters 626 LC system (Waters, MA) on a 10/30 HiLoad Superdex 75 column at a flow rate of 0.5 mL/min. The column was calibrated with dextran blue (Sigma) for the void volume and the protein standards thyroglobulin (670 kDa), bovine ?-globulin (158 kDa), chicken ovalbumin (44 kDa), equine myoglobin (17.8 kDa), and vitamin B12 (1.35 kDa, all standards from Biosil2000, Bio-Rad). Fractions were analysed by silver stained SDS-PAGE (data not shown) and PCL-fractions were concentrated and used for further analysis. The Ras lipoprotein shows oligomerisation under non denaturating conditions (upper figure, 2nd signal).