**Electronic Supplementary Information to:**

# Rhodopsins carrying structurally modified chromophores – the 'making of', modelling, and their light-induced reactivity

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## Contents

## Synthesis of 9-demethyl retinal (2)

see Fig. S1 for overview

*i) 3-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-propenoate (**8**)*

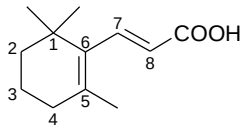
β-Ionon (47 g (0.24 mol) were brought to 0°C. Within 30 min, 200 ml (0.82 mol) a pre-cooled solution (13 %) of sodium hypochloride was added dropwise, followed by 60 ml methanol (20 min). The solution is stirred for 2 h at 0°C. the reaction is then acidified (half-conc. phosphoric acid) and extracted with ether. Excess of ß-Ionon is removed upon rising the pH (10% solution of NaOH), thus bringing the reaction into the aqueous solution. Re-acidification and extraction with ether yields the reaction product (16.5 g) which is purified by crystallization (ethanol/water). Yield: 12.3 g (64 mmol, 27 %) as colorless needles.

**UV** (ethanol): (7*E*) $\lambda_{max}$ = 277 nm

**IR** (film): (7*E*) 3400-2200 br, ν(O-H); 1687 vs, ν(C=O); 1631 s, ν(C=C)

**$^1$H-NMR** (250 MHz, $CDCl_3$): (7*E*)

| 1,1- | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 7-H | 8-H | -OH |
|---|---|---|---|---|---|---|---|
| 1.06 s | 1.46 m | 1.60 m | 2.06 t | 1.77 d | 5.83 d | 7.54 d | ~ 11.6 |




| J(3,4) | J(5-$CH_3$,7) | J(7,8) | J(8,OH) |
|---|---|---|---|
| 6.13 | 0.45 | 16.15 | 0.65 |

*ii) 3-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-propenol (**9**)*

12.2 g (63 mmol) of compound (**2**), dissolved in 100 mL of di-ethylether, were added dropwise at 0°C to a suspension of Lithiumaluminum hydride (3.8 g, 95 mmol, 1.5 eq) in di-ethylether (15 mL). Following, the reaction mixture was refluxed for 2 h. Excess of $LiAlH_4$ is hydrolyzed by conz. Ammonium chloride-solution. The reaction mixutre is filtered, phase-separated and the ether removed. Vacuum distillation 96 °C (0.4 Torr) yields 6.3 g (34 mmol, 54 %) of alcohol (**9**) as colorless liquid (pure 7*E*-isomer).

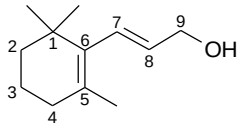
**UV** (ethanol): (7*E*) $\lambda_{max}$ = 234 nm

**IR** (film): (7*E*) 3321 vs, br, ν(O-H); 1643 w, ν(C=C); ~1400 br, δ(O-H); 1011 vs, ν(C-0)

**$^1$H-NMR** (400 MHz, $CDCl_3$): (7*E*)

| 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 7-H | 8-H | 9-$H_2$ |
|---|---|---|---|---|---|---|---|
| 0.98 s | 1.43 m | 1.58 m | 1.96 t | 1.66 d | 6.10 d | 5.59 dt | 4.18 d |

| J(3,4) | J(5-$CH_3$,7) | J(7,8) | J(8,9) |
|---|---|---|---|
| 6.15 | 0.79 | 15.90 | 15.97 |




*iii) 3-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-propenal (**10**)*

Pre-activated (3 h bei 105 °C, 0.2 Torr) $MnO_2$ (59 g, 680 mmol) is poured into an ice-cold solution of (**9**), 6.2 g (34 mmol) in 150 ml di-chloro methane. Reaction control (thin layer chromatography, n-hexane/ethyl acetate 85/15) identifies after 50 min complete conversion into the aldehyde. After filtration, extraction with di-ethyl ether and di-chloro methane and removal of organic solvents remained a slightly yellow liquid (5.6 g) which is purified by distillation (110 °C, 0.2 Torr). Final yield are 5.0 g (28 mmol, 82 %) von **10** as colorless liquid (pre 7*E*-isomer).

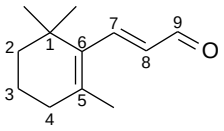
**UV** (ethanol): (7*E*) $\lambda_{max}$ = 292 nm

**IR** (film): (7*E*) 2725 m; 1688 vs, ν(C=O); 1605 vs, ν(C=C)

**$^1$H-NMR** (250 MHz, $CDCl_3$): (7*E*)

| 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 7-H | 8-H | 9-H |
|---|---|---|---|---|---|---|---|
| 1.09 s | 1.49 m | 1.61 m | 2.09 t | 1.79 s | 7.25 d | 6.17 dd | 9.51 d |

| J(3,4) | J(7,8) | J(8,9) |
|---|---|---|
| 6.10 | 16.10 | 7.80 |




See Fig. S2 for $^1$H-NMR spectrum

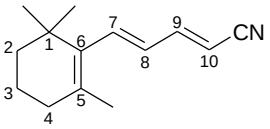
*iv) 5-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2,4-pentadiene nitrile (**11**)*

Sodium hydride (1.6 g, 40 mmol, 1.4 eq, 60 % suspension in oil) and 6.0 g (34 mmol, 1.2 eq) $C_2$-phosphonate are allowed to react with 5.0 g (28 mmol, 1 eq) of compound **10**. Chromatographic purification (silica, n-hexane/di-ethyl ether 90/10) yielded 4.9 g (24 mmol, 86 %) of nitrile **11** as colorless crystals (1:2 ratio of 9*Z*- and all-*E*-isomer).

**IR** (film): (isomeric mixture) 2211 vs, ν(C≡N); 1606 vs, ν(C=C)

**$^1$H-NMR** (250 MHz, $CDCl_3$):

| | 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 7-H | 8-H | 9-H | 10-H |
|---|---|---|---|---|---|---|---|---|---|
| 9*Z* | 1.03 s | 1.45 m | 1.58 m | 2.03 t | 1.75 s | 6.58 d | 6.84 m | 6.58 d | 5.09 d |
| all-*E* | 1.01 s | | | | 1.71 s | 6.54 d | 6.16 dd | 7.02 dd | 5.24 d |




| | J(3,4) | J(7,8) | J(8,9) | J(9,10) |
|---|---|---|---|---|
| 9*Z* | ~5.9 | 5.97 | * | 10.67 |
| all-*E* | | 15.63 | 10.92 | 15.91 |

*, not to be determined

*v) 5-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2,4-pentadienal (**12**)*

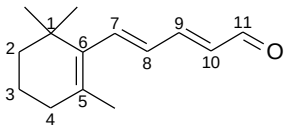
Nitrile **11 (**4.8 g (24 mmol, 1 eq) is reduced with 34 ml (34 mmol, 1.4 eq) of a 1 M DIBAH-solution to obtain aldehyde **12**. Chromatographic purification (silica, n-hexane/di-ethyl ether 90/10) yielded 4.0 g (20 mmol, 83 %) of aldehyde **12** (9*Z*/all-*E* 1:4).

**UV** (ethanol): (all-*E*) $\lambda_{max}$ = 326 nm

**IR** (film): (all-*E*) 2826 w, sh, 2729 w; 1682 vs, ν(C=O); 1610 vs, ν(C=C)

**$^1$H-NMR** (250 MHz, $CDCl_3$):

| | 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 7-H | 8-H | 9-H | 10-H | 11-H |
|---|---|---|---|---|---|---|---|---|---|---|
| 9*Z* | 1.05 s | ~1.47 m | ~1.60 m | ~2.05 t | 1.75 s | 6.59 d | 6.93-7.08 * | | 5.82 dd | 10.15 d |
| all-*E* | 1.05 s | 1.46 m | 1.59 m | 2.05 t | 1.75 d | 6.71 d | 6.35 dd | 7.15 dd | 6.11 dd | 9.55 d |




| | J(3,4) | J(5-$CH_3$,7) | J(7,8) | J(8,9) | J(9,10) | J(10,11) |
|---|---|---|---|---|---|---|
| 9*Z* | ** | ** | 14.4 | * | 9.80 | 8.00 |
| all-*E* | 6.2 | 0.69 | 15.63 | 10.89 | 15.22 | 8.00 |

*, not resolved;

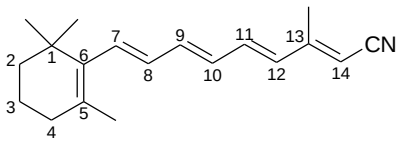
**, a mixture of both isomers was measured, coupling constants could not be calculated.

*vi) 3-Methyl-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraennitrile (**13**)*

Sodium hydride (1.1 g, 28 mmol, 1.4 eq, 60 % suspension in oil) and 5.1 g (24 mmol, 1.2 eq) of $C_5$-phosphonate were allowed to react with **4**.0 g (20 mmol, 1 eq) of aldehyde **12**. Chromatographic purification (silica, n-hexane/di-ethyl ether 95/5) yielded 3.9 g (14.6 mmol, 74 %) of an isomeric mixture of nitrile **13**

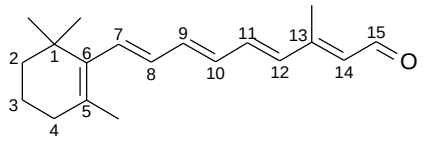
**IR** (film): (mixture of isomers) 2007 vs, ν(C≡N); 1582 s, br, ν(C=C)

**$^1$H-NMR** (250 MHz, $CDCl_3$): unseparated mixture of isomers Isomerengemisch. Indicative signal: singulet of 14-H, at 5.14 ppm for major component (all-*E* isomer).



*vii) 3-Methyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenal (9-demethyl retinal, **2**)*

Nitril **13** (3.8 g, 14.2 mmol, 1 eq) was reduced with 20 mL (20 mmol, 1.4 eq) of a 1 M DIBAH-solution to generate aldehyde **2** reduziert. Purification by silica (n-hexane/ethyl acetate, 90/10) yielded 2.8 g (10 mmol, 73 %) of a dark-red viscous oil, comprised of a mixture of at least four isomers.




**IR** (film): (all-*E*) 1654 vs, ν(C=O); 1577 s, ν(C=C)

**Mass spectrometry** (EI, 85 °C), m/z (%): (all-*E*) 270 (100, $M^+$, $C_{21}H_{26}O$), 255 (22, $[M - CH_3]^+$)

(high res., EI): (all-*E*) ber.: 270.198365, gef.: 270.198399

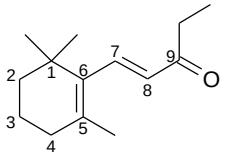
$^{13}$**C-NMR** (101 MHz, broad band decoupled, acetone-$d_6$): (all-*E*) 191.22 (C-15); 154.89 (C-13); 139.40 (C-9); 138.17 (C-6); 137.37 (C-11); 135.21 (C-7); 135.14 (C-12); 134.07 (C-8); 131.92 (C-5); 131.62 (C-10); 129.82 (C-14); 40.47 (C-2); 34.75 (C-1); 33.82 (C-4); 28 (1,1-$CH_3$); 21.90 (5-$CH_3$); 19.77 (C-3); 12.91 (13-$CH_3$)

## Synthesis of 9-ethyl retinal (3)

*i) 5-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-4-penten-3-on (**14**) and (2-Methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-4-penten-3-on (**15**)*

β-Ionon (11.5 g, 60 mmol) in THF was added within 15 min at -60 °C to 30 ml (60 mmol) of a 2 M solution of lithium di-isopropylamine dissolved in 50 ml THF. The reaction mixture was stirred for another 15 min, after which time a solution of 8.5 g (60 mmol) of methyl iodide in 20 mL THF was added over 15 min. After this addition, the reaction mixture was brought to 0 °C and stirred for 3 h. Working-up started with addition of 30 mL of a saturated ammonium chloride solution, followed by searation and extraction of the aqueous layer (three times) with di-ethyl ether. The combined organic phases were neutralized and removed. The remaining red-colored oil was twice chromatographically purified (n-hexane/di ethyl ether 90/10), yielding 3.65 g (18 mmol, 30 %) of an orange-colored oil (**14**). In addition, 2.06 g (9.4 mmol) double alkylated ß-Ionon **15** was obtained.

a) Compound **14** (monoalkylated product)




**UV** (ethanol): (7*E*) $\lambda_{max}$ = 295 nm

**IR** (film): (7*E*) 1716 w, sh, 1694 s, sh, 1673 vs, ν(C=O); 1609 s, ν(C=C)

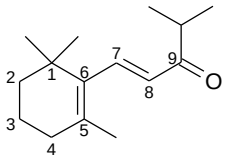
**Mass spectrometry** (EI, -20°C), m/z (%): (7*E*) 206 (13, $M^+$, $C_{14}H_{22}O$), 191 (100, $[M - CH_3]^+$), 177 (7, $[M - C_2H_5]^+$), 149 (13, $[M - COC_2H_5]^+$)

$^1$**H-NMR** (250 MHz, $CDCl_3$): (7*E*)

| 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 7-H | 8-H | 9-$CH_2$ | 9-$CCH_3$ |
|---|---|---|---|---|---|---|---|---|
| 1.08 s | 1.45 m | 1.59 m | 2.04 t | 1.74 d | 7.29 d | 6.11 d | 2.59 q | 1.11 t |

| J(3,4) | J(5-$CH_3$,7) | J(7,8) | J(9-$CH_2$,9-$CCH_3$) |
|---|---|---|---|
| 6.2 | 0.74 | 16.35 | 7.35 |

b) Verbindung **15** (double alkylated product)




**UV** (ethanol): (7*E*) $\lambda_{max}$ = 295 nm

**IR** (film): (7*E*) 1716 w, sh, 1694 s, sh, 1673 vs, ν(C=O); 1609 s, ν(C=C)

**Mass spectrometry** (EI, -20°C), m/z (%): (7*E*) 220 (17, $M^+$,$C_{15}H_{24}O$), 205 (100, [M - $CH_3$]$^+$), 177 (55, [M - $C_3H_7$]$^+$), 149 (18, [M - $COC_3H_7$]$^+$)

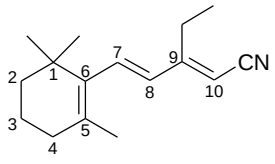
**$^1$H-NMR** (250 MHz, $CDCl_3$): (7*E*)

| 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 7-H | 8-H | 9-CH | 9-$CCH_3$ |
|---|---|---|---|---|---|---|---|---|
| 1.05 s | 1.46 m | 1.60 m | 2.04 t | 1.75 d | 7.33 dd | 6.17 d | 2.82 sep | 1.12 |

| J(3,4) | J(7,8) | J(5-$CH_3$,7) | J(9-CH,9-$CCH_3$) |
|---|---|---|---|
| 6.4 | 16.18 | 0.80 | 6.90 |

*ii) 3-Ethyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadiene nitrile (**16**)*

$C_2$-Phosphonate (4.3 g, 25 mmol, 1.4 eq) were reacted with 1.13 g sodium hydride (28 mmol, 1.6 eq, 60 % in oil); the yild was then reacted with 3.6 g (18 mmol, 1 eq) of ketone **14**. Purification over silica (n-hexane/di-ethyl ether 90/10) yielded 3.6 g (16 mmol, 90 %) of nitrile **16** as a yellow colored oil. $^1$H-NMR analysis determined a 9*Z*/all-*E* ratio of 1:1.




**IR** (film): (mixture of isomers) 2210 vs, ν(C≡N)

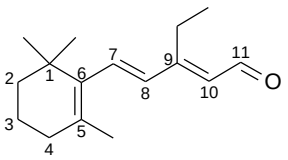
**$^1$H-NMR** (400 MHz, $CDCl_3$):

| | 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 9-$CH_2$ | 9-$CCH_3$ | 7-H | 8-H | 10-H |
|---|---|---|---|---|---|---|---|---|---|---|
| 9*Z* | 1.02 s | 1.46 m | 1.60 m | 2.03 t | 1.73 s | 2.42 dq | 1.14 t | 6.61 s | | 5.08 s |
| all-*E* | 1.00 s | 1.46 m | 1.60 m | 2.00 t | 1.68 d | 2.61 q | 1.18 t | 6.58 d | 5.99 d | 5.08 s |

| | J(3,4) | J(5-$CH_3$,7) | J(9-$CH_2$,X) | J(9-$CH_2$,9-$CCH_3$) | J(7,8) |
|---|---|---|---|---|---|
| 9*Z* | 5.8 | - | 1.17 | 7.45 | 0 |
| all-*E* | 6.0 | 0.81 | - | 7.60 | 16.19 |

*iii) 3-Ethyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadienal (**17**)*

Reduction of nitrile **16** (3.5 g, 15 mmol, 1 eq) to form aldehyde **17** was accomplished by reaction with 21 ml (21 mmol, 1.4 eq) of a 1 M DIBAH-solution. Purification on silica (n-hexane/di-ethyl ether 85/15) yielded 2.65 g of aldehyde **17** (11.4 mmol, 76 %) as a 9*Z*- / all-*E*-isomer mixture in as ratio of 2:3.




**IR** (film): (mixture of isomers) 1668 vs, ν(C=O); 1609 s, ν(C=C)

**$^1$H-NMR** (250 MHz, $CDCl_3$)*:

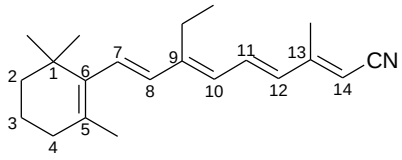
| | 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 9-$CH_2$ | 9-$CCH_3$ | 7-H | 8-H | 10-H | 11-H |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 9*Z* | 1.02 s | ~1.45 m | ~1.60 m | ~2.03 t | 1.73 d | 1.16 dq | 2.45 t | 6.58 d | 6.87 d | 5.86 d | 10.13 d |
| all-*E* | 1.03 s | | | | 1.70 d | 1.24 q | 2.79 t | 6.80 d | 6.06 d | 5.85 d | 10.08 d |

| | J(3,4) | J(5-$CH_3$,7) | J(9-$CH_2$ ,X) | J(9-$CH_2$,9-$CCH_3$) | J(7,8) | J(10,11) |
|---|---|---|---|---|---|---|
| 9*Z* | 6.1 | 0.83 | 0.92 | 7.46 | 16.09 | 8.04 |
| all-*E* | 6.1 | 0.84 | - | 7.64 | 16.27 | 8.21 |

** as an isomeric mixture was measured, signals of protons at positions 2, 3 and 4 could not unambigously be assigned.*

*iv) 7-Ethyl-3-methyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraene nitrile (**18**)*

Double bond chain extension to generate **18** was accomplished by using sodium hydride (0.63 g, 15.7 mmol, 1.4 eq, 60 % in oil) and 2.92 g (13.4 mmol, 1.2 eq) $C_5$-Phosphonat to generate the $C_5$-yilde which was allowed to react with aldehyde **17** (2.6 g, 11.2 mmol, 1 eq). Chromatographic purification on silica (n-hexane/di-ethyl ether 90/10) yielded 3.08 g (10.4 mmol, 93 %) of nitrile **18** as a light-reddish colored oil; the product was composed of a mixture of at least four isomers.



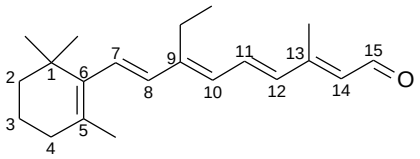

**IR** (film): (mixture of isomers) 2207 vs, ν(C≡N); 1583 s, ν(C=C)

**$^1$H-NMR** (400 MHz, $CDCl_3$): mixutre of isomers, non separated.

Indicative for the successful reaction is the presence of proton at $C_{14}$ as a singulet at 5.15 ppm (position for the all-*E* isomer, major component)

*v) 7-Ethyl-3-methyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenal (9-Ethylretinal, **3**)*

Nitrils **18** (3.0 g, 10.2 mmol, 1 eq) were reduced with 14 ml (14 mmol, 1.4 eq) of a 1 M DIBAH-solution, yielding 2.45 g (8.5 mmol, 83 %) of an isomeric mixture of 9-ethyl retinal (**3**).




**$^{13}$C-NMR** (101 MHz, BB-decoupled, acetone-$d_6$): (all-*E*) 191.22 (C-15); 155.13 (C-13); 147.88 (C-9); 138.56 (C-6); 136.69 (C-8); 136.05 (C-12); 132.81 (C-11); 130.41 (C-5); 129.83, 129.76, 129.53 (C-7/ C-10/ C-14); 40.27 (C-2); 34.93 (C-1); 33.54 (C-4); 29 (1,1-$CH_3$); 20.71 (9-$CH_2$); 19.88 (C-3); 14.90 (9-$CCH_3$); 13.03 (13-$CH_3$)

**Mass spectrometry** (EI, 80 °C), m/z (%): (all-*E*) 298 (100, $M^+$, $C_{21}H_{30}O$), 283 (12, $[M - CH_3]^+$), 269 (20, $[M - C_2H_5]^+$)

(high resolution, EI): (all-*E*) ber.: 298.2297, gef.: 298.2298

## Synthesis of 9-isopropyl retinal (4)

*i) 2-Methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-4-penten-3-on (**15**)*

see above.

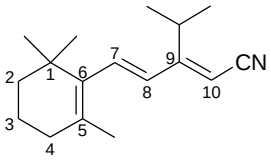
*ii) 3-(1-Methylethyl)-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadiennitrile (**19**)*

$C_2$-Phosphonate (1.9 g, 5.4 mmol, 1.2 eq) is converted into ist yild by reaction with sodium hydride (0.3 g, 13 mmol, 1.4 eq, 60 % in oil) and is allowed to react with Keton **15** (2.0 g, 9 mmol) to yield nitrile **19**. Chromatographic purification on silica (n-hexane/di-ethyl ether 90/10) led to 1.71 g (7.03 mmol, 78 %) of product.

**IR** (film): (mixture of isomers) 2211 vs, ν(C≡N); 1607 s, 1575 s, ν(C=C)

**$^1$H-NMR** (250 MHz, $CDCl_3$)*:

| | 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 9-CH | 9-$CCH_3$ | 7-H | 8-H | 10-H |
|---|---|---|---|---|---|---|---|---|---|---|
| 9*Z* | 1.00 | ~1.46m | ~1.54 m | ~2.02 t | 1.73 d | 2.86 sep | 1.14 d | 6.65 d | 6.53 d | 5.08 s |
| all-*E* | 1.00 | | | | 1.67 d | 3.17 sep | 1.18 d | 6.58 d | 5.44 d | 5.22 s |




| | J(3,4) | J(5-$CH_3$,7) | J(9-CH,9-$CCH_3$) | J(7,8) |
|---|---|---|---|---|
| 9*Z* | 5.8 | 0.71 | 6.89 | 16.04 |
| all-*E* | 5.8 | 0.65 | 6.93 | 16.32 |

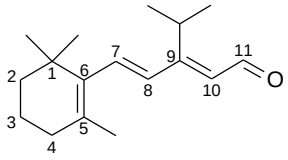
** a mixture of isomers was measured, accordingly, an assignment of protons at positions 2, 3, and 4 was not possible.*

*iii) 3-(1-Methylethyl)-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadienal (**20**)*

Nitrile **19** (1.7 g, 7 mmol, 1 eq) is reduced to yield aldehyde **20** by reaction with 10 ml (10 mmol, 1.4 eq) of a 1 M DIBAH-solution. Chromatography on silica (n-hexane/di-ethyl ether 90/10) yielded 1.09 g (4.4 mmol, 63 %) of aldehyde **20** as a reddish-colored oil, composed of a mixture of all-*E* and 9*Z*-Isomer with ca. 60 % of 9*Z*-isomer.

**$^1$H-NMR** (250 MHz, $CDCl_3$):

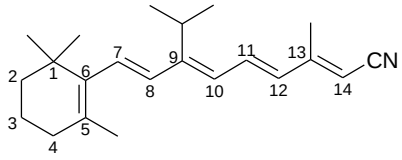
| | 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 9-CH | 9-$CCH_3$ | 7-H | 8-H | 10-H | 11-H |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 9*Z* | 1.02 s | 1.46 m | 1.60 m | 2.02 t | 1.73 d | 2.74 sep | 1.14 d | 6.44 d | 6.53 d | 5.91 d | 10.02 d |
| all-*E* | 1.01 s | 1.46 m | 1.61 m | 2.01 t | 1.70 d | 3.62 sep | 1.19 d | 6.71 d | 6.06 d | 6.09 d | 10.09 d |




| | J(3,4) | J(5-$CH_3$,7) | J(9-CH,9-$CCH_3$) | J(7,8) | J(10,11) |
|---|---|---|---|---|---|
| 9*Z* | 6.3 | 0.35 | 6.84 | 15.98 | 7.88 |
| all-*E* | 6.3 | 0.75 | 6.90 | 16.05 | 8.20 |

*iv) 7-(1-Methylethyl)-3-methyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraene nitrile (**21**)*

Aldehyde **20** (1.0 g, 4.06 mmol, 1 eq) was brought to reaction with the ylid of $C_5$-phosphonate (1.3 g, 5.7 mmol, 1.4 eq), generated from 0.16 g (6.5 mmol, 1.6 eq, 60 % in oil) of sodium hydride. Chromatographic purification on silica (n-hexane/ di-ethyl ether 90/10) yielded nitrile **21** (1.25 g, 4.05 mmol, 99 %) as a mixture of at least three stereoisomers.



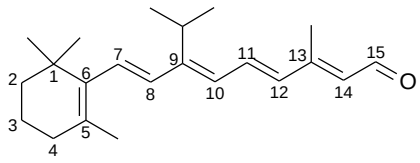

**IR** (film): (mixture of isomers) 2207 vs, ν(C≡N); 1568 s, ν(C=C)

**$^1$H-NMR** (400 MHz, $CDCl_3$): mixture of isomers.

Indicative for successful reaction is position of singulet of 14-H at 5.14 ppm for the dominant 9*Z*-9-Isopropyl nitrile.

*v) 7-(1-Methylethyl)-3-methyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenal (9-Isopropylretinal, **4**)*

Nitrile **21** ((1.2 g, 3.9 mmol, 1 eq) was reduced to yield 9-isopropyl retinal (**4**) using 5.4 ml (5.4 mmol, 1.4 eq) of a 1 M DIBAH-solution. Upon purification on silica (n-hexane/di-ethyl ether 90/10) 1.13 g (3.6 mmol, 93 %) of a dark-red viscous oil were obtained composed of a mixture of 9*Z*,13*Z* (12 %), 13*Z* (6%), 9*Z* (53 %) and all-*E*-isomers (29 %).




**Mass spectrometry:** (EI, 75 °C), m/z (%): (all-*E*) 312 (100, $M^+$, $C_{22}H_{32}O$), 297 (10, $[M - CH_3]^+$), 269 (34, $[M - C_3H_7]^+$), high resolution (EI): (all-*E*) ber.: 312.2453, gef.: 312.2451

**$^{13}$C-NMR** (101 MHz, BB-decoupled, $CDCl_3$): (all-*E*) 191.25 (C-15); 155.30 (C-13); 151.54 (C-9); 138.76 (C-6); 136.02 (C-12); 133.60 (C-8); 132.58 (C-11); 130.28 (C-7); 130.22 (C-5); 129.72 (C-14); 125.21 (C-10); 40.23 (C-2); 34.87 (C-1); 33.46 (C-4); 29 (1,1-$CH_3$); 21.97, 21.88 (5-$CH_3$/ 9-**C**$H_2$/ 9-C**C**$H_3$); 19.90 (C-3); 13.06 (13- $CH_3$)

## Synthesis of 10-methyl-13-demethylretinal (5)

*i) 2,3-Dimethyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadiene nitrile (**22**)*

Di-isopropyl amine 13.7 ml (98 mmol, 1.95 eq), dried on calcium hydride and dissolved in THF (100 mL, freshly distilled over $LiAlH_4$) were given into a baked-out 500 mL three-neck flask (internal thermometer, gas inlet, and dropping funnel) and

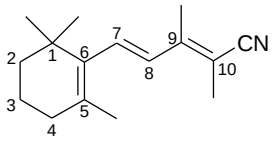
cooled under Ar stram to -20 °C. Into this solution, were added 59.4 ml (95 mmol, 1.9 eq) of a 1,6 M n-hexane solution of n-butyl lithium within 15 min. After complete addition, the reaction mixture was brought to -70 °C, and within another 15 min a solution of 3.4 ml (48 mmol, 0.95 eq) of dry propionitrile in 10 ml abs. THF was added, forming a white suspension, which was stirred again for 15 min at -70 °C. Following, a solution of 6.9 ml (48 mmol, 0.95 eq) di-ethyl chlorophosphate in 10 ml THF is added. This reaction mixture is now brought to 0 °C, and is adjoined (over a time period of 60 min) with a solution of ß-Ionon (10.2 ml, 50 mmol, 1 eq) in 10 ml abs. THF. Final stirring of this solution was for 2 h at ambient temperature.

Work-up: the reaction mixture was carefully hydrolized with saturated brine (ca. 50 ml). Separated aqueous phase was extracted three times with ether, combined organic phases were washed neutral (brine) and dried. After removal of organic solvents, the reddish, viscous oil was purified by flash chromatography (8 x 30 cm, silica, n-hexane/di-ethyl ether 90/10), yielding 8.96 g (39.1 mmol, 78 %) of nitrile **22** containing ca. 90 % of all-*E*-isomer. Further purification was not done.

**IR** (film): (all-*E*) 2204 vs, ν(C≡N); 1612 s, ν(C=C)

**$^1$H-NMR** (500 MHz, acetone-$d_6$): (all-*E*); only dominant signals of all-*E* isomer were assigned.

| 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 9-$CH_3$ | 10-$CH_3$ | 7-H | 8-H |
|---|---|---|---|---|---|---|---|---|
| 1.02 s | 1.47 m | 1.61 m | 2.02 t * | 1.71 d | 2.18 q | 1.97 q | 6.59 d | 6.50 d |




| J(5-$CH_3$,7) | J(9-$CH_3$,10-$CH_3$) | J(7,8) |
|---|---|---|
| 0.61 | ~1.35 | 16.09 |

** this signal is admixted by a second peak from another isomer; coupling constant could not be determined.*

*ii) 2,3-Dimethyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadienal (**23**)*

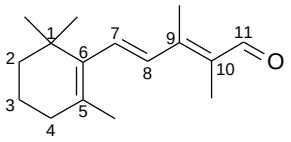
Nitrile **22** (8.9 g, 39 mmol, 1 eq) was reduced with DIBAH (62 mmol, 1.6 eq, 1 M solution in n-hexane) to yield aldehyde **23**. After chromatographic purification (n-hexane/di-ethyl ether 80/20) 7.6 g (33 mmol, 84 %) of product were obtained (mixture of 9*Z*- and all-*E*-isomer, ratio 9:1).

**IR** (film): (all-*E*) 2204 vs, ν(C≡N); 1612 s, ν(C=C)

**Mass spectrometry** (EI, 30°C), m/z (%): (all-*E*) 232 (77, $M^+$, $C_{16}H_{24}O$); 217 (21, $[M - CH_3]^+$); 123 (45, $[M - C_9H_{15}]^+$, Ionone-ring); 107 (100, $[M - C_7H_9O]^+$, polyene chain after loss of ringand -2 $H^+$)

**$^1$H-NMR** (500 MHz, $CDCl_3$):

| | 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 9-$CH_3$ | 10-$CH_3$ | 7-H | 8-H | 11-H |
|---|---|---|---|---|---|---|---|---|---|---|
| 9*Z* | 1.02 s | 1.47 m | 1.61 m | 2.02 t | 1.71 s | 2.09 q | 1.85 q | 6.45 d | 6.96 d | 10.26 s |
| all-*E* | 1.03 s | 1.47 m | 1.61 m | 2.02 t | 1.75 s | 2.29 q | 1.85 q | 6.71 d | 6.65 d | 10.26 s |




| | J(3,4) | J(5-$CH_3$,7) | J(9-$CH_3$,10-$CH_3$) | J(7,8) |
|---|---|---|---|---|
| 9*Z* | 6.26 | 0.83 | 0.8 | 15.90 |
| all-*E* | 6.55 | - | 1.15 | 16.12 |

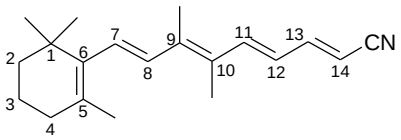
$^{13}$**C-NMR** (126 MHz, BB-Entkopplung, $CDCl_3$): (all-E) 191.75 (C-11); 149,37 (C-9); 137.72 (C-6); 134.61 (C-7); 132.37 (C-8); 132.28 (C-5 und C-10); 39.51 (C-2); 34.20 (C-1); 33.21 (C-4); 28.95 (1,1-$CH_3$); 21.81 (5-$CH_3$); 19.05 (C-3); 12.58 (9-$CH_3$); 10.62 (10-$CH_3$)

*iii) 6,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraene nitrile (**24**)*

Aldehyde **23** (2.0 g, 8.4 mmol, 1 eq) were brought to reaction with the ylid $C_4$-phosphonate (2.54 g, 12.5 mmol, 1.5 eq), pre-formed by reaction with 8.2 ml (13.1 mmol, 1.55 eq) of a 1.6 M n-BuLi-solution. From chromatographic purification (flash-column, silica, n-hexane/di-ethyl ether 80/20) a fraction was isolated, composed of at least three stereoisomers. Yield: 1.15 g (4.1 mmol, 49 %).

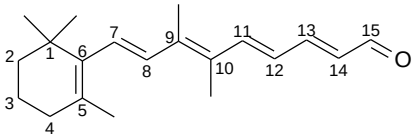
**IR** (film): (mixture of isomers) 2209 vs, ν(C≡N)

$^{1}$**H-NMR** (250 MHz, $CDCl_3$): mixture of isomers, indicative for successful reaction: 5.31 d and 5.09 d (14-H)




*iv) 6,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenal (10-Methyl-13-demethylretinal **6**)*

Nitril **24** (1.15 g, 4.1 mmol, 1 eq) was reduced with DIBAH (8.2 ml, 8.2 mmol, 2 eq, 1M solution). Chromatographic purification (silica, n-hexane/di-ethyl ether 90/10) yielded 1.11 g (3.9 mmol, 95 %) of **6**, composed of a mixture of four isomers.




**Mass spectrometry** (EI, 70 °C), m/z (%): (all-*E*) 284 (100, $M^+$, $C_{20}H_{28}O$), 269 (19, $[M - CH_3]^+$) (high resolution, EI): (all-*E*) ber.: 284.2140, gef.: 284.2143

$^{13}$**C-NMR** (126 MHz, BB-decoupled, $CDCl_3$): (all-*E*) 193.59 (C-15); 153.55 (C-13); 142.60 (C-11); 138.40, 138.27 (C-6/C-9); 132.51 (C-8); 131.21 (C-7); 130.51 (C-5); 130.15 (C-14); 129.60 (C-10);

125.78 (C-12); 39.59 (C-2); 34.23 (C-1); 33.16 (C-4); 28.99 (1,1-$CH_3$); 21.84 (5-$CH_3$); 19.16 (C-3); 14.16 (9-$CH_3$); 13.79 (10-$CH_3$)

## Synthesis of 10-methylretinal (6)

*i) 3,6,7-Trimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraene nitrile (**25**)*

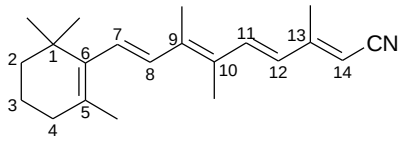
2,3-Dimethyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadienal (**23**, 2.0 g (8.4 mmol, 1 eq) were allowed to react with the ylid of $C_5$-phosphonate (2.74 g, 12.5 mmol, 1.5 eq) pre-formed by reaction with a 1.6 M BuLi solution (8.2 mL, 13.1 mmol, 1.55 eq). Crude reaction product (3.17 g) was purified on a silica flash-column (n-hexane/di ethyl ether 80/20), yielding compound **25** (2.21 g, 7.53 mmol, 89 %) as a mixture of at least two stereoisomers with more than 90 % all-*E*.

**IR** (film): (> 90 % all-*E*) 2209 s, ν(C≡N)

**$^1$H-NMR** (400 MHz, $CDCl_3$):

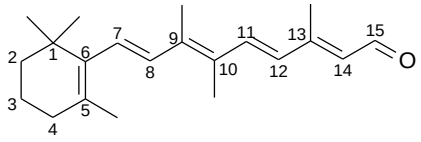
| 1,1-$CH_3$ | 2-$H_2$ | 3-$H_2$ | 4-$H_2$ | 5-$CH_3$ | 9-$CH_3$ | 10-$CH_3$ | 13-$CH_3$ | 7-H | 8-H | 11-H | 12-H | 14-H |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.02 s | ~2 t | 1.61 m | 1.47 m | 1.73 s | 1.92 s | 2.00 s | 2.21 s | 6.25 d | 6.67 d | 7.28 d | 6.28 d | 5.19 |

| J(7,8) | J(11,12) |
|---|---|
| 15.93 | 15.55 |




*ii) 3,6,7-Trimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenal, (10-Methylretinal, **6**)*

10-Methyl retinal is formed by reaction of nitrile **25** (2.21 g, 7.53 mmol, 1 eq) with 15 ml (15 mmol, 2 eq) of a 1 M DIBAH-solution. Chromatographic purification (silica, n-hexane/di ethyl ether 85/15) yield 1.62 g (5.4 mmol, 72 %) of compound **6** with more than 80% consisting of the all-*E*-isomer.




**Mass spectrometry:** (EI, 85 °C), m/z (%): (all-*E*) 298 (100, $M^+$, $C_{21}H_{30}O$), 283 (21, $[M - CH_3]^+$), high resolution, EI: (all-*E*) ber.: 298.2297, gef.: 298.2303

$^{13}$**C-NMR** (126 MHz, BB-decoupled, acetone-$d_6$): (all-*E*) 181.5 (C-15); 146.28 (C-13); 129.70 (C-6); 127.82 (C-9); 126.41 (C-11); 124.37 (C-8); 122.63 (C-12); 121.39 (C-7); 121.17, 121.04 (C-5/ C-10); 120.70 (C-14); 30.89 (C-2); 25.43 (C-1); 24.21 (C-4); 20 (1,1-$CH_3$); 12.63 (5-$CH_3$); 10.45 (C-3); 4.90, 4.61 (9-$CH_3$/ 10-$CH_3$); 3.67 (13-$CH_3$)

Fig. S1: reaction scheme for synthesis of 9-demethyl retinal (**2**)

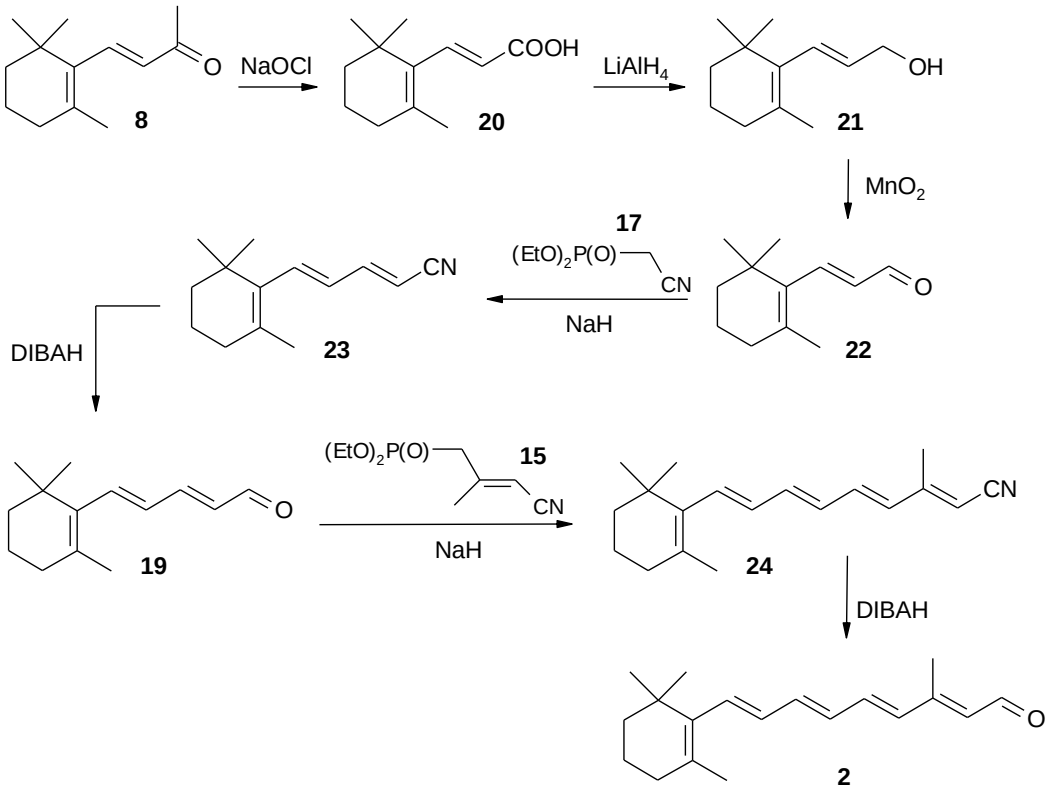
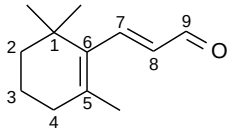


Fig. S2: $^{1}$H-NMR spectrum for (**10**)



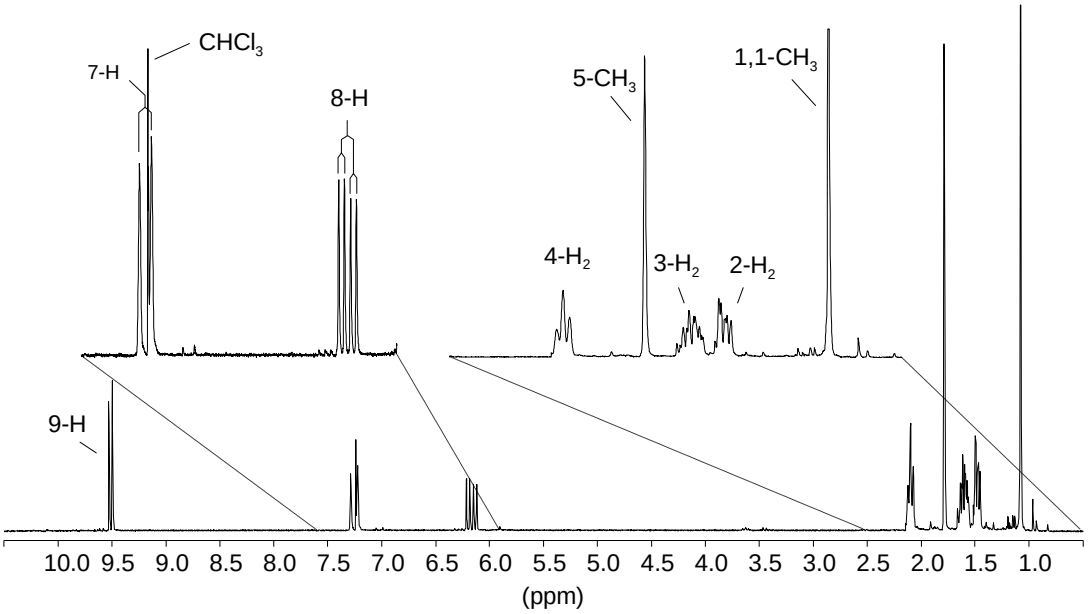
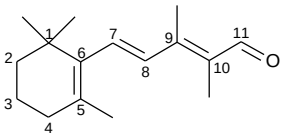



Fig. S3: $^{1}$H-NMR spectrum for (**23**)



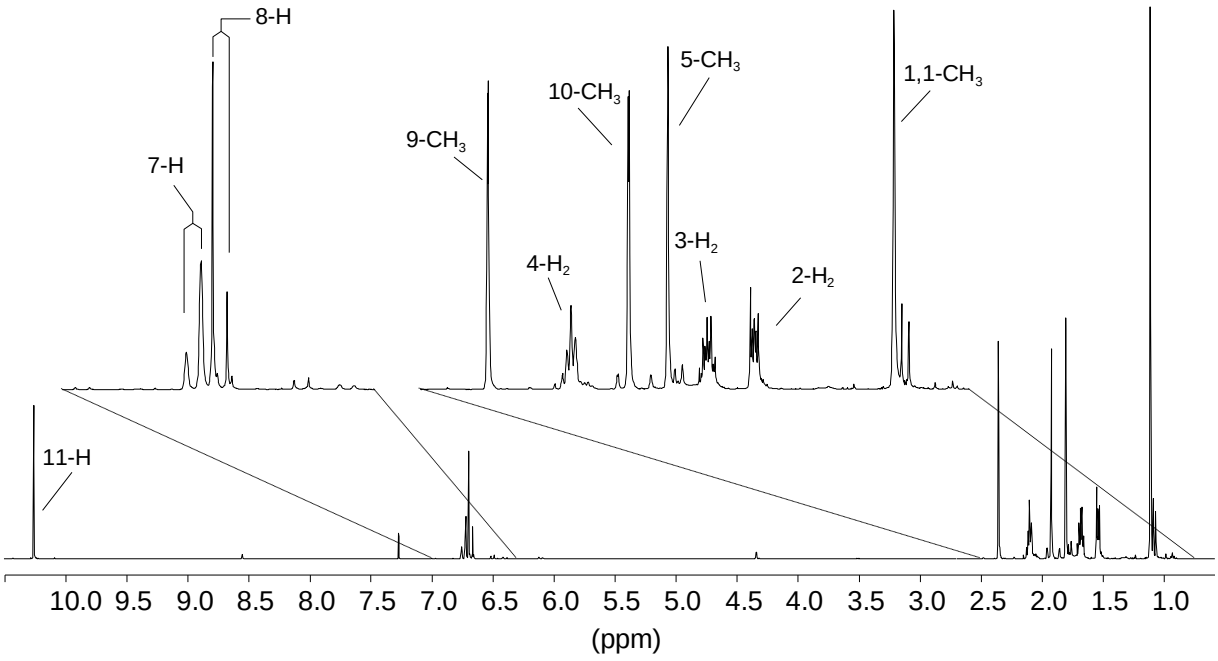



Fig. S4: Time course for light-induced isomerization, starting with 100% of all-*trans* isomer

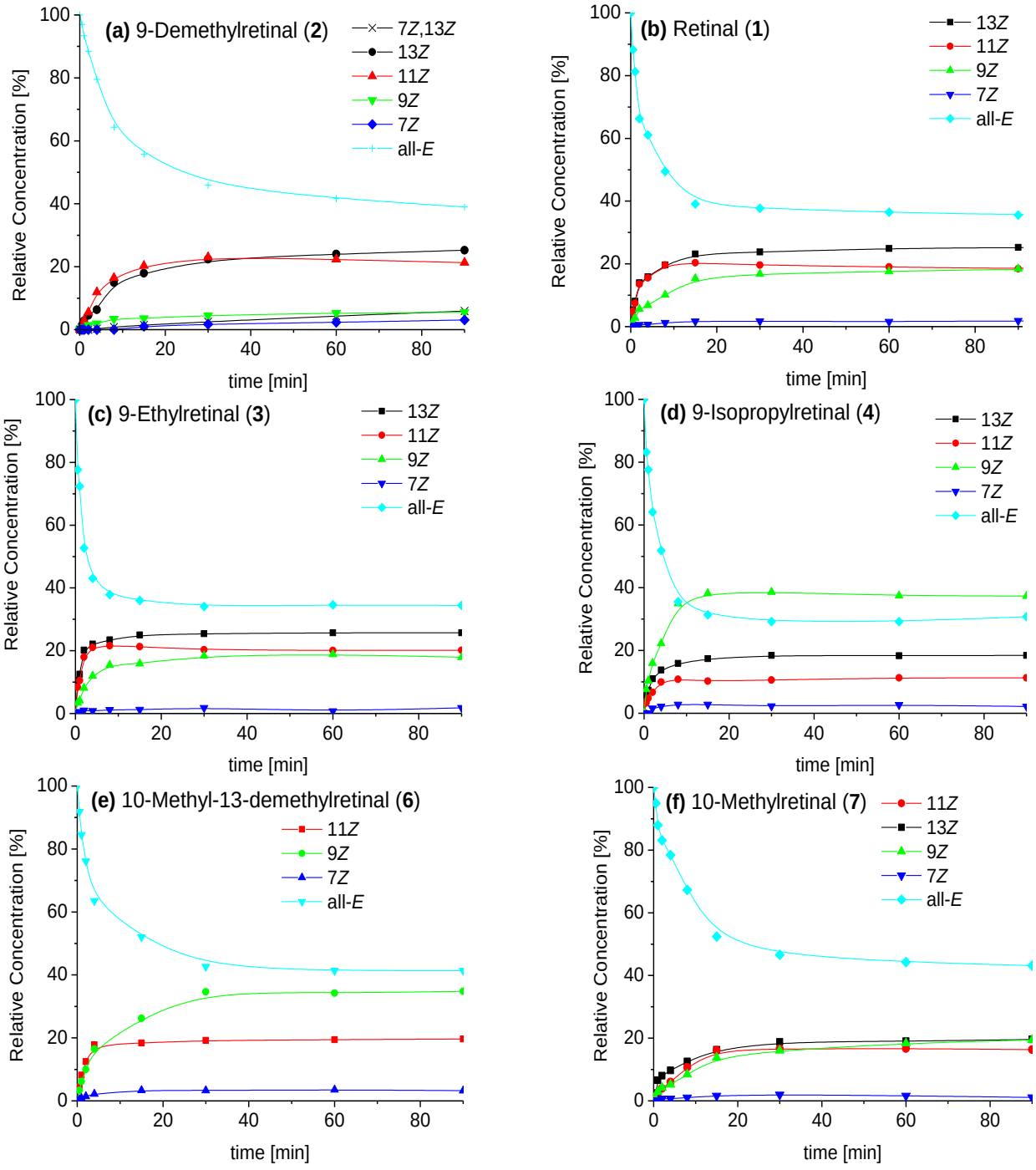


Table S1: Absorption maxima of isomers of retinal and retinal derivatives (in n-hexane/di-ethylether, 9:1) underlined: dominant peak; * literature data: Liu, R.S.H. and Asato, A.E. (1984) Tetrahedron. 40, 1931-1969.

| | 9-dm | Retinal | 9-Et | 9-Is | MD | 10-Me | 13-dm |
|---|---|---|---|---|---|---|---|
| 7*Z*,13*Z* | 315 | 357* | | | | | |
| 7*Z* | 352 | 364 | 362 | 364 | 354 | 360 | 358 |
| 9Z,11*Z* | | 352* | 365 | | | | |
| 9*Z*,13*Z* | | 359* | 361 | 354 | | 362 | |
| 9*Z* | 360 | 362 | 361 | 354 | 366 | 366 | 362 |
| 11*Z*,13*Z* | | | 300 | | | | |
| 11*Z* | 250, 358 | 252, 366 | 254, 366 | 254, 360 | 254, 358 | 273, 344 | 246 ,368 |
| 13*Z* | 250, 358 | 257, 364 | 254, 366 | 254, 360 | | 370 | 368 |
| all-*E* | 362 | 370 | 370 | 364 | 371 | 373 | 368 |

Table S2: Assembly kinetics of opsin with 11-*cis* isomers of retinal and retinal derivates [s]

| | 9-dm | Retinal | 9-Et | 13-dm | MD | 10-Me |
|---|---|---|---|---|---|---|
| $\tau_1$ | 87 | 101 | 156 | 81 | 109 | 373 |
| $\tau_2$ | 533 | 387 | 910 | 1055 | 1672 | 1700 |
| $\tau_3$ | 7503 | 3491 | 4804 | 5234 | 5005 | 7850 |
| $\tau_4$ | | | 15.5 h | | | |

Table S3: Absorption maxima of retinal derivative oxims and chromoprotein and calculated opsin shift

| | $\lambda_{max}$ [nm] | | Opsin-shift |
|---|---|---|---|
| | Oxim | Chromoprotein | [$cm^{-1}$] |
| 9-dm | 358 | 464 | 1384 |
| Retinal | 364 | 500 | 2522 |
| WT | " | 500 | " |
| 9-Et | 364 | 500 | 2727 |
| 9-Is | 354 | 490 | 2422 |
| 13-dm | 364 | 500 | 2727 |
| MD | 364 | 504 | 2580 |
| | 348 (11*Z*) | | |
| 10-Me | 366 | 508 | 2636 |

Fig. S5: Rhodopsin reconstitution using 11-*cis* retinal (pH 7, 20°C)

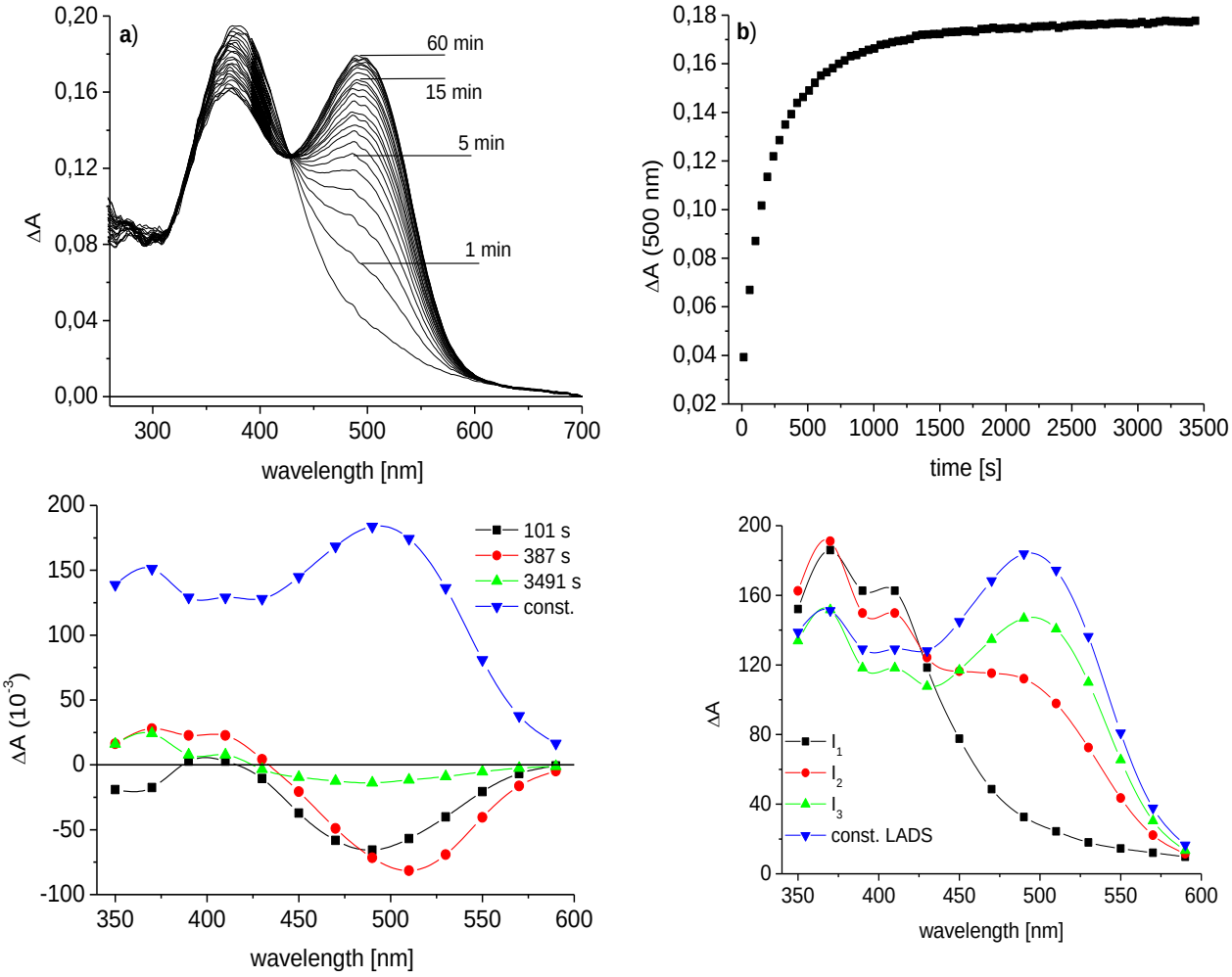


Fig. S6: Rhodopsin reconstitution using 11-*cis* 10-methyl-13-demethyl (MD) retinal (pH 7, 20°C)

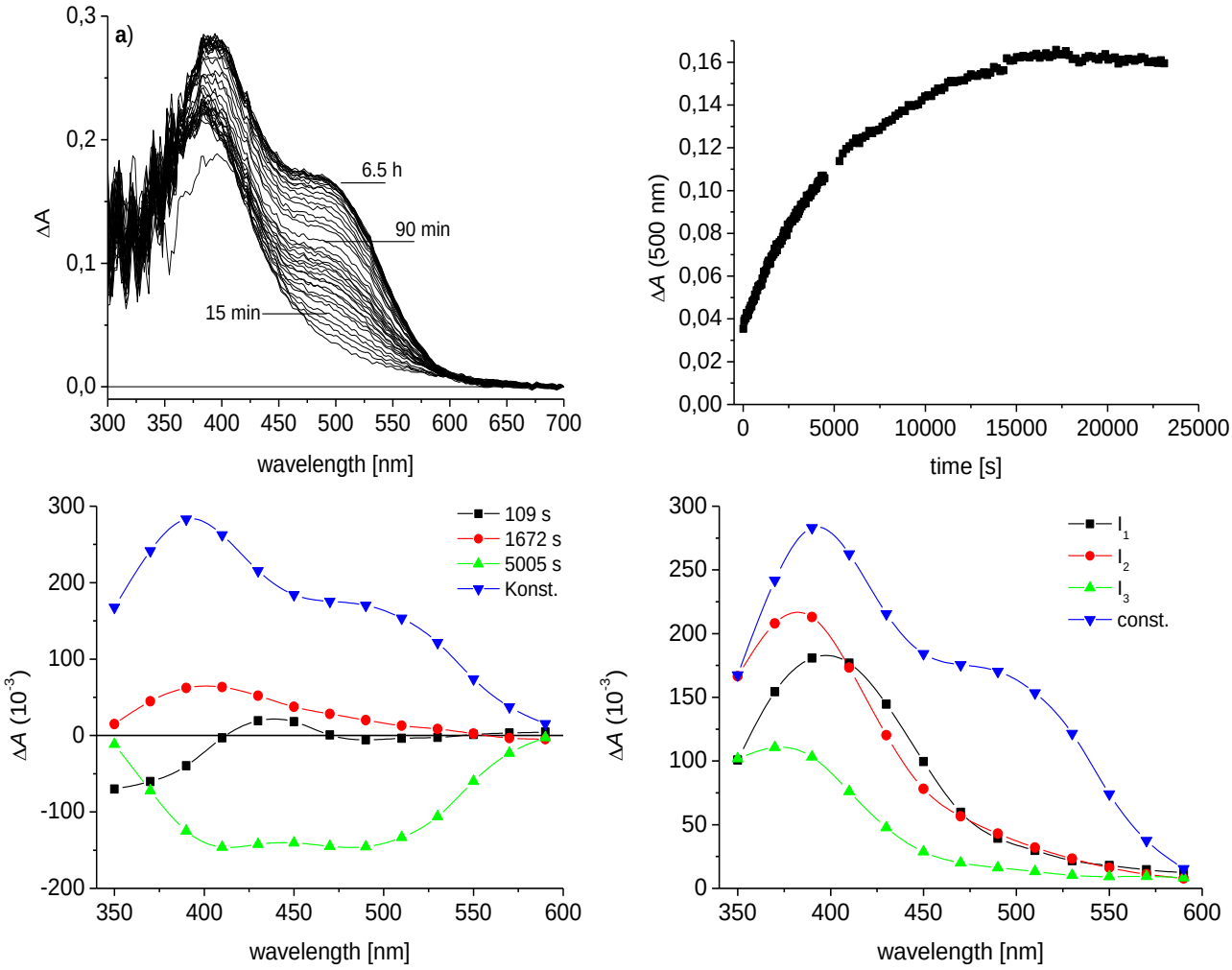


Fig. S7: Bleaching experiments of rhodopsins carrying (top) 9ipr- and (bottom) the MD retinal derivatives

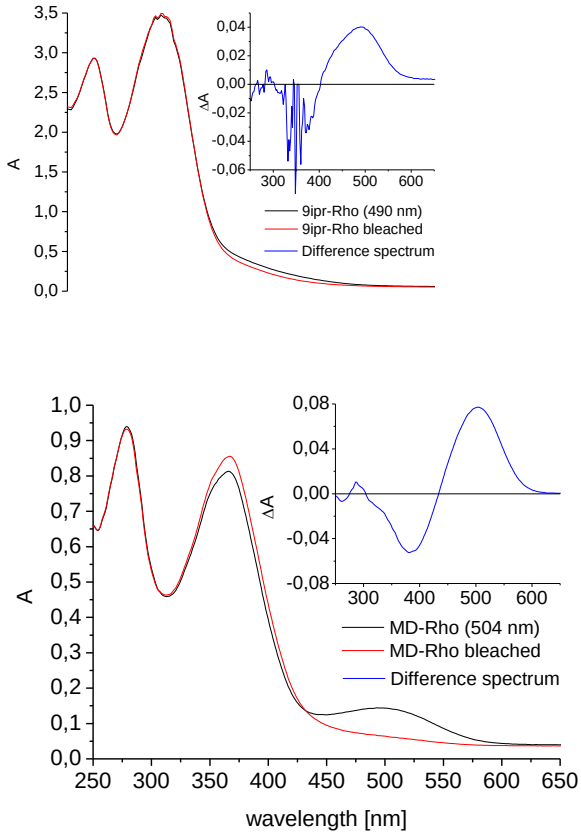
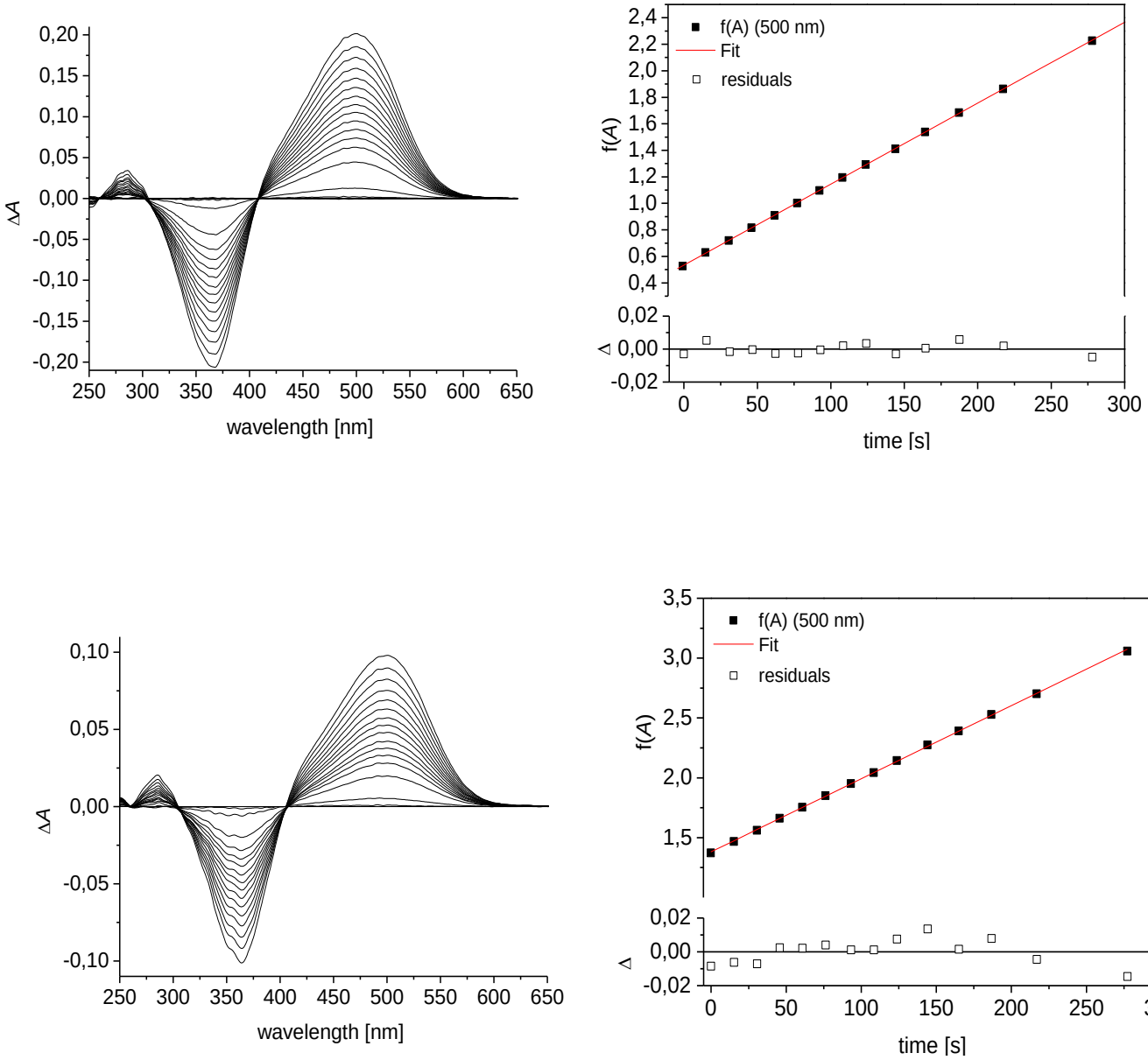
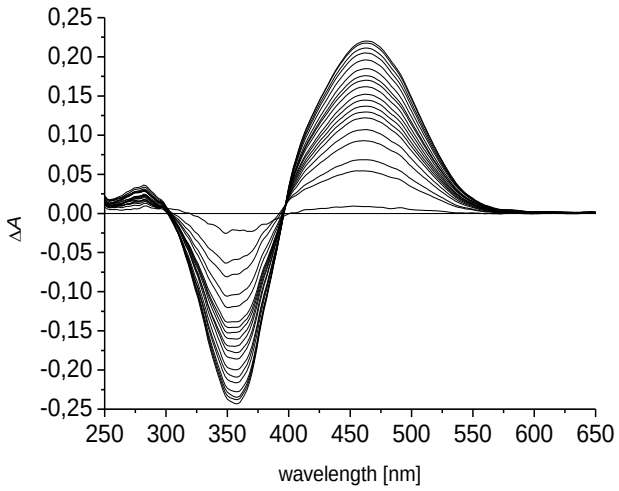
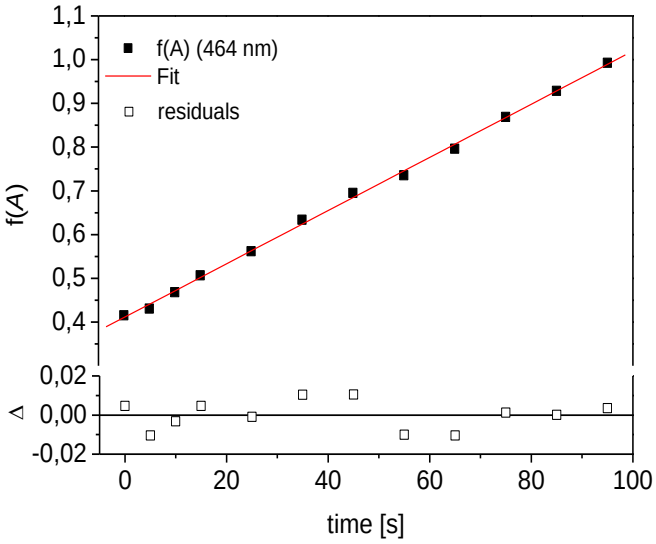
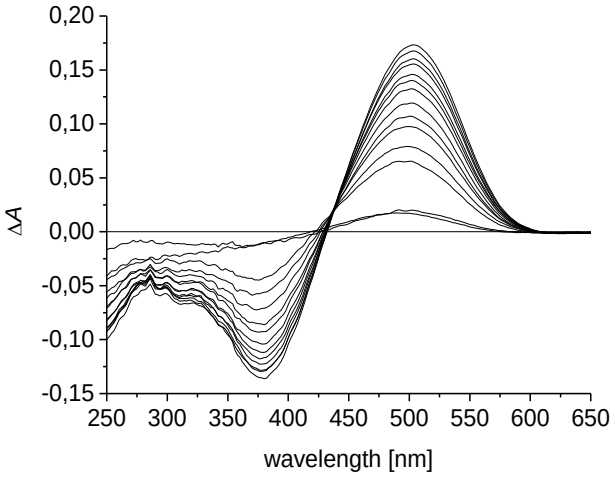


Fig. S8: Determination of quantum yields for (top) native rhodopsin, (second) rhodopsin reconstituted from 11-*cis* retinal and opsin, (third) 11-*cis* 9et retinal and opsin, and (fourth) 11-*cis* 13dm retinal and opsin




0,25
0,20
0,15
0,10
0,05
0,00
-0,05
-0,10
-0,15
-0,20
-0,25
ΔA
250 300 350 400 450 500 550 600 650
wavelength [nm]


1,1
1,0
0,9
0,8
0,7
0,6
0,5
0,4
f(A)
f(A) (464 nm)
Fit
residuals
0,02
0,00
-0,02
Δ
0 20 40 60 80 100
time [s]


0,20
0,15
0,10
0,05
0,00
-0,05
-0,10
-0,15
ΔA
250 300 350 400 450 500 550 600 650
wavelength [nm]

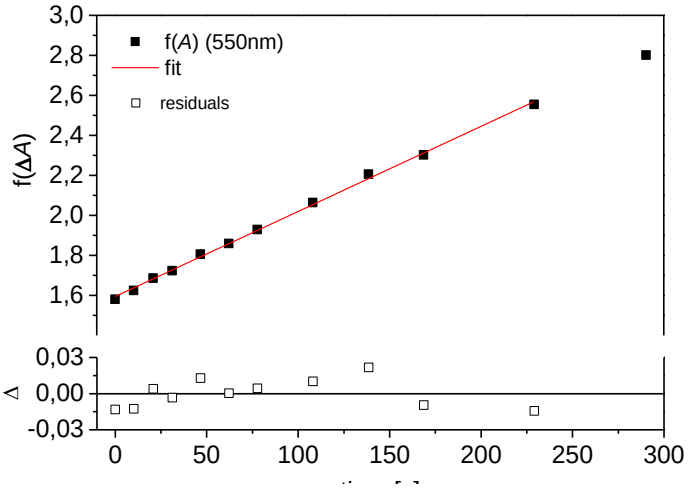

3,0
2,8
2,6
2,4
2,2
2,0
1,8
1,6
f(ΔA)
f(A) (550nm)
fit
residuals
0,03
0,00
-0,03
Δ
0 50 100 150 200 250 300

Fig. S9: Thermal instability (dark reaction) of MD-assembled rhodopsin against 10 mM hydroxylamine: left, series of absorbance spectra; right, absorbance changes determined between consecutively following spectra (spectrum $X_{i+5}$) – (spectrum $X_i$)

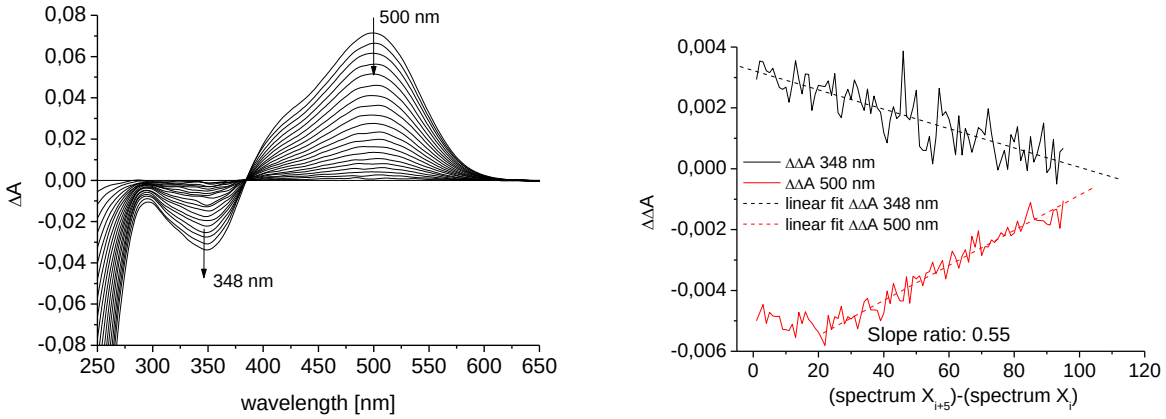



Fig. S10: Detergent effect on the stability of 13dm-assembled rhodopsin in the dark: left, 13dm-rhodopsin in DDM and LDAO (3%) without addition of hydroxylamine, immediate and after 90 min (strong loss of chromophore); right, stability of 13dm-rhodopsin against hydroxylamine in the dark over 3h (no change).