

A light-gated OmpG hybrid

Supporting Information

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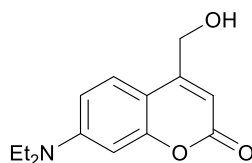
1. Syntheses

Compounds **1**¹, **2**², **5**² and **7**¹ were prepared using reported procedures. All reactions were performed using dry solvents which were purchased from *Sigma Aldrich Co.* and *Acros*. Reagents were purchased from *Sigma Aldrich Co.*, *Roth* and *TCI* and were used without further purification. For flash chromatography silica gel 60 by *Macherey-Nagel* was used. TLC analyses were performed on aluminum plates coated with silica gel 60 F 254 (*Merck*).

NMR spectra for the characterisation of the compounds were recorded on *Bruker* AVIII-HD500 (500 MHz), AV400 (400 MHz) and DRX600 (600MHz) instruments equipped with a N₂ cooled cryogenic probe head using DMSO-d⁶ and CDCl₃ as solvent. HRMS spectra were recorded using a *Thermo Scientific MALDI LTQ Orbitrap*.

Synthesis of compound 3

7-(Diethylamino)-4-(hydroxymethyl)-2H-chromene-2-one¹ (**2**)



C₁₄H₁₇NO₃
Mol. Wt.: 247.29 g/mol

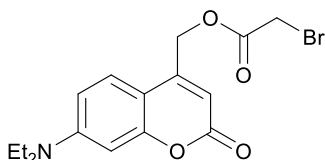
4.63 g (20.0 mmol, 1.0 eq.) 7-Diethylamino-4-methylcoumarin (**1**) were dissolved in 120 mL *p*-xylene and 3.33 g (30.0 mmol, 1.5 eq.) SeO₂ were slowly added. The reaction mixture was stirred at 155 °C overnight, filtered to remove SeO₂ and the solvent was removed *in vacuo*. To 130 mL EtOH 380 mg (10 mmol, 0.5 eq.) NaBH₄ were slowly added. The reaction mixture was stirred for 4 h at room temperature, acidified with 20 mL HCl (1.0 M) and 50 mL H₂O were added and extracted three times with 50 mL CH₂Cl₂. The combined organic layers were washed with 50 mL brine, dried over Na₂SO₄ and the solvent was removed *in vacuo*. Purification was accomplished via flash chromatography (CH₂Cl₂:acetone 5:1). The solvent was removed *in vacuo* to afford 7-(diethylamino)-4-(hydroxymethyl)-2H-chromene-2-one (**2**) (1.11 g, 4.5 mmol, 22%) as a light green-yellow solid.

¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm]: 7.42 (d, 1H, *J* = 9.00 Hz, aromatic-*H*), 6.66 (dd, 1H, *J* = 9.05 Hz, aromatic-*H*), 6.52 (d, 1H, *J* = 2.55 Hz, aromatic-*H*), 6.06 (s, 1H, aromatic-*H*), 5.50 (t, 1H, *J* = 5.60 Hz, OH), 4.66 (dd, 2H, *J* = 5.60 Hz, CH₂OH), 3.41 (q, 4H, *J* = 7.05 Hz, 2 x CH₂CH₃), 1.11 (t, 6H, *J* = 7.00 Hz, 2 x CH₂CH₃).

¹³C-NMR (125 MHz, DMSO-*d*₆) δ [ppm]: 161.1, 156.9, 155.6, 150.2, 125.1, 108.5, 105.7, 103.9, 96.8, 59.0, 43.9, 12.3.

HRMS(MALDI): *m/z* calcd. for C₁₄H₁₈NO₃ [M+H]⁺ 248.12812,
found 248.12866 (Δ*m* = 0.00054, error 2.18 ppm).

(7-(Diethylamino)-2-oxo-2*H*-chromene-4-yl)methyl-2-bromoacetate² (3)



C₁₆H₁₈BrNO₄
Mol. Wt.: 368.22 g/mol

100 mg (0.40 mmol, 1.0 eq) 7-(Diethylamino)-4-(hydroxymethyl)-2*H*-chromen-2-on (**2**) were dissolved in 7 mL dry toluene. 50 μL (0.62 mmol, 1.5 eq.) dry pyridine and 54 μL (0.62 mmol, 1.5 eq.) bromoacetyl bromide were added. The reaction mixture was stirred at room temperature overnight. The precipitate was filtered and washed with EtOAc. The organic solvent was removed *in vacuo*. Purification was accomplished via flash chromatography (EtOAc). The solvent was removed *in vacuo* to afford (7-(diethylamino)-2-oxo-2*H*-chromene-4-yl)methyl-2-bromoacetate (**3**) (136 mg, 0.37 mmol, 93%) as yellow solid.

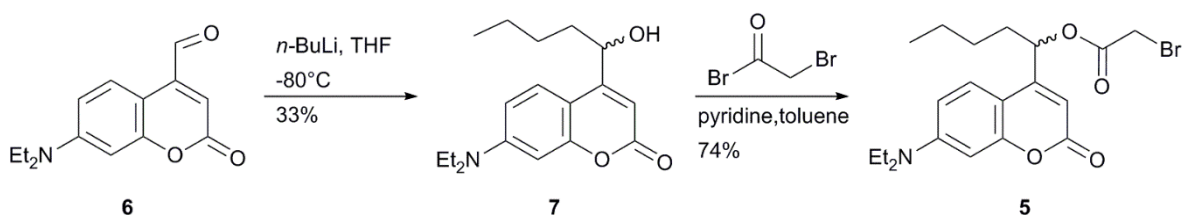
¹H-NMR (400 MHz, DMSO-*d*₆) δ [ppm]: 7.46 (d, 1H, *J* = 9.04 Hz, aromatic-*H*), 6.69 (dd, 1H, *J* = 9.04 Hz, aromatic-*H*), 6.55 (d, 1H, *J* = 2.52 Hz, aromatic-*H*), 6.07 (s, 1H, aromatic-*H*), 5.39 (s, 2H, CH₂O), 4.34 (s, 2H, CH₂Br), 3.43 (q, 4H, *J* = 8.00 Hz, 2 x CH₂CH₃), 1.12 (t, 6H, *J* = 8.00 Hz, 2 x CH₂CH₃).

¹³C-NMR (100 MHz, DMSO-*d*₆) δ [ppm]: 166.8, 160.5, 155.8, 150.5, 149.9, 125.5, 108.7, 105.1, 105.0, 96.8, 62.5, 44.0, 26.8, 12.3.

HRMS(MALDI): *m/z* calcd. for C₁₆H₁₉BrNO₄ [M+H]⁺ 368.04920,
found 368.04892 (Δ*m* = 0.00028, error 0.76 ppm).

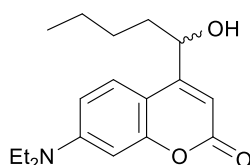
Synthesis of Compound 5

Compound **6** (7-(diethylamino)-2-oxo-2H-1-benzopyran-4-carboxaldehyde) was synthesized in analogy to compound **2** according to literature¹.



Supporting Figure 1: reaction scheme for the synthesis of compound **5**.

7-(Diethylamino)-4-(2-hydroxypentyl)-2H-chromene-2-one (**7**)



$\text{C}_{18}\text{H}_{25}\text{NO}_3$
Mol. Wt.: 303.40 g/mol

123 mg (0.50 mmol, 1.0 eq.) 7-(Diethylamino)-2-oxo-2H-1-benzopyran-4-carboxaldehyde (**6**) were dissolved in 2.0 mL THF and cooled to -80°C . Then, 300 μL (0.75 mmol, 1.5 eq., 2.5 M in n -hexane diluted in 3 mL THF) $n\text{-BuLi}$, prechilled to -80°C , were added and the reaction mixture stirred for 60 minutes at -80°C and at room temperature overnight. The reaction was stopped by addition of 3 mL saturated NH_4Cl solution. After addition of EtOAc the organic layer was washed with water and brine, dried over MgSO_4 and the solvent evaporated under vacuum. Purification was accomplished via flash chromatography (CH_2Cl_2 :acetone – 33:1) to yield 7-(diethylamino)-4-(2-hydroxypentyl)-2H-chromene-2-on (**7**)(50 mg, 0.17 mmol, 33%) as a yellow solid.

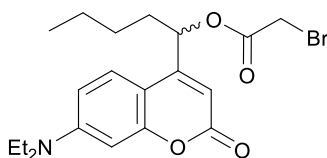
R_f : 0.6 (CH_2Cl_2 :acetone – 6:1).

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ [ppm]: 7.41 (d, 1H, $J = 9.06$ Hz, aromatic- H), 6.57 (dd, 1H, $J = 9.06$ Hz, $J = 2.58$ Hz, aromatic- H), 6.51 (d, 1H, $J = 2.58$ Hz, aromatic- H), 6.24 (s, 1H, aromatic- H), 5.02 – 4.94 (m, 1H, CHOH), 3.41 (q, 4H, $J = 7.14$ Hz, 2x CH_2CH_3), 2.07 (d, 1H, $J = 3.84$ Hz; OH); 1.89 – 1.83 (m, 1H, $\text{CHCH}_{2,a}$), 1.77 – 1.70 (m, 1H, $\text{CHCH}_{2,b}$), 1.56 – 1.48 (m, 1H, $\text{CH}_2\text{CH}_{2,a}\text{CH}_2$), 1.48 – 1.43 (m, 1H, $\text{CH}_2\text{CH}_{2,b}\text{CH}_2$), 1.42 – 1.32 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.21 (t, 6H, $J = 7.08$ Hz, 2x CH_2CH_3), 0.92 (t, 3H, $J = 7.32$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_3$).

$^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ [ppm]: 162.7, 158.5, 156.5, 150.3, 125.0, 108.5, 106.3, 105.2, 98.0, 70.1, 44.7, 44.7, 37.0, 27.9, 22.5, 14.0, 12.5, 12.5.

HRMS(MALDI): m/z calcd. for $C_{18}H_{26}NO_3$ $[M+H]^+$ 304.19072,
found 304.19076 ($\Delta m=0.00004$, 0.1 ppm).

(7-(Diethylamino)-2-oxo-2H-chromene-4-yl)pentyl-2-bromoacetate² (5)



$C_{20}H_{26}BrNO_4$
Mol. Wt.: 424.34 g/mol

50 mg (0.17 mmol, 1.0 eq.) 7-(diethylamino)-4-(2-hydroxypentyl)-2H-chromene-2-one (**7**) were dissolved in 2 mL toluene and 22 μ L pyridine (0.25 mmol, 1.5 eq.) were added. After 5 min 0.20 mL (0.25 mmol, 1.5 eq.) bromoacetyl bromide were added and the reaction mixture stirred at room temperature for 16 h. 15 mL EtOAc and 15 mL water were added and the organic phase washed with 10 mL water and 10 mL brine. Purification was accomplished via flash chromatography (CH:EtOAc – 5:1). The solvent was removed *in vacuo* to afford (7-(diethylamino)-2-oxo-2H-chromene-4-yl)pentyl-2-bromoacetate (**5**) (53 mg, 0.12 mmol, 74%) as yellow oil.

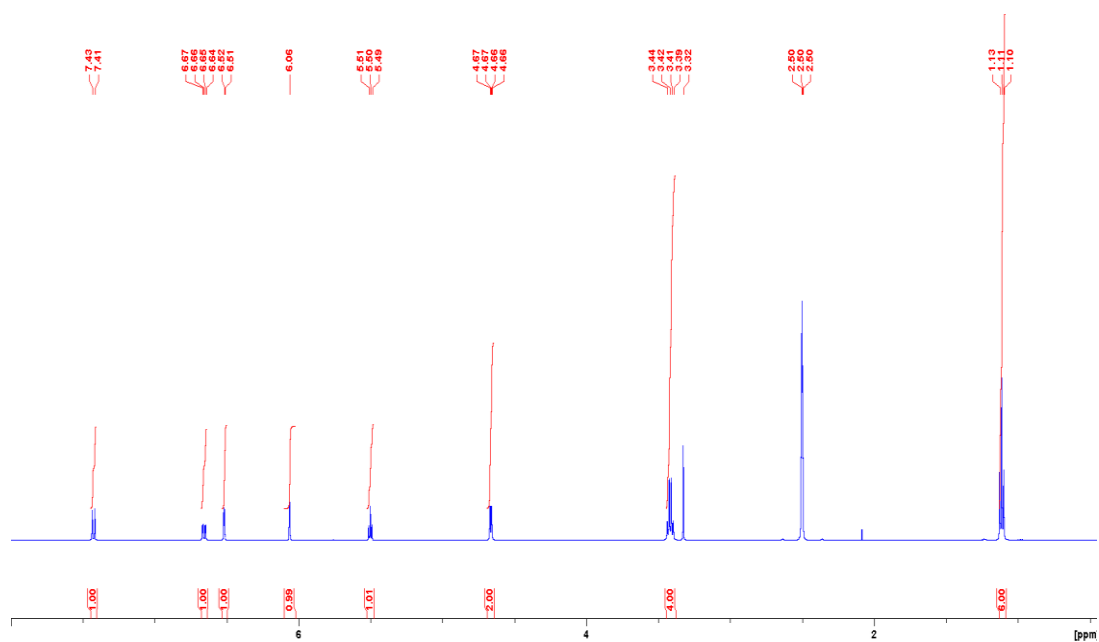
R_f: 0.4 (CH:EE – 4:1).

¹H-NMR (600 MHz, CDCl₃) δ [ppm]: 7.40 (d, 1H, $J = 9.06$ Hz, aromatic-*H*), 6.60 (dd, 1H, $J = 9.06$ Hz, $J = 1.98$ Hz, aromatic-*H*), 6.52 (d, 1H, $J = 2.12$ Hz, aromatic-*H*), 6.10 (s, 1H, aromatic-*H*), 5.99 (t, 1H, $J = 6.36$ Hz, CH), 3.91 (d, 1H, $J = 12.36$ Hz, CH_{2,a}Br), 3.89 (d, 1H, $J = 12.42$ Hz, CH_{2,b}Br), 3.41 (q, 4H, $J = 7.08$ Hz, 2x CH₂CH₃), 1.95 – 1.90 (m, 2H, CHCH₂CH₂), 1.46 – 1.31 (m, 4H, CH₂CH₂CH₂CH₃), 1.21 (t, 6H, $J = 7.08$ Hz, 2x CH₂CH₃), 0.90 (t, 3H, $J = 7.20$ Hz, CH₂CH₂CH₃).

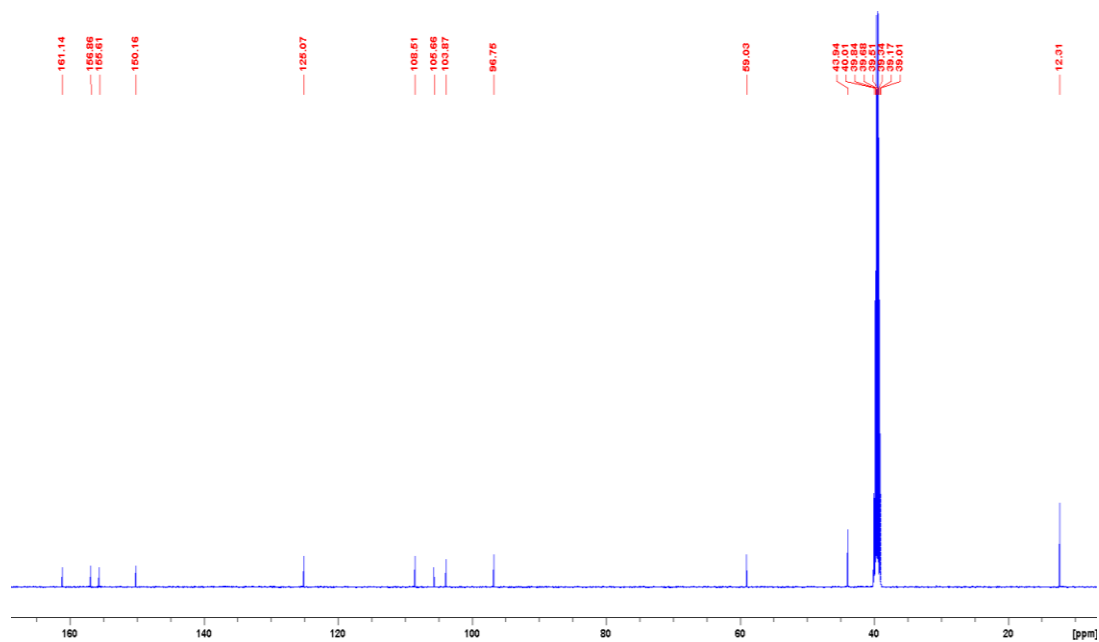
¹³C-NMR DEPT (150 MHz, CDCl₃) δ [ppm]: 124.8, 108.8, 105.6, 98.2, 72.9, 44.9, 44.9, 34.5, 27.5, 25.4, 22.3, 13.9, 12.5, 12.5.

HRMS(MALDI): m/z calcd. for $C_{20}H_{27}BrNO_4$ $[M+H]^+$ 424.11180,
found 424.11134 ($\Delta m=0.00046$, 1.1 ppm).

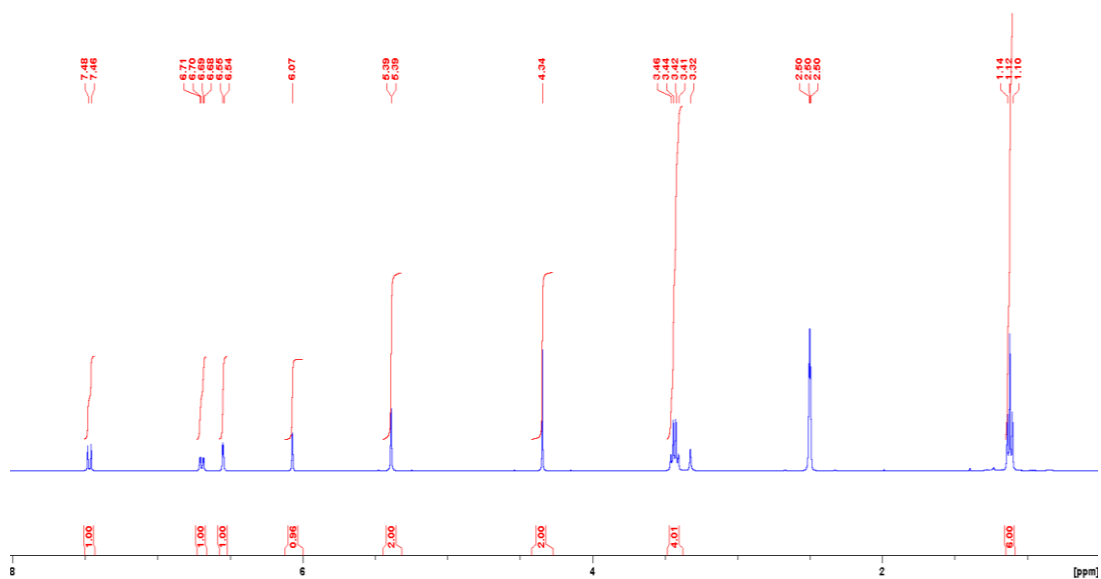
2. NMR-Spectra



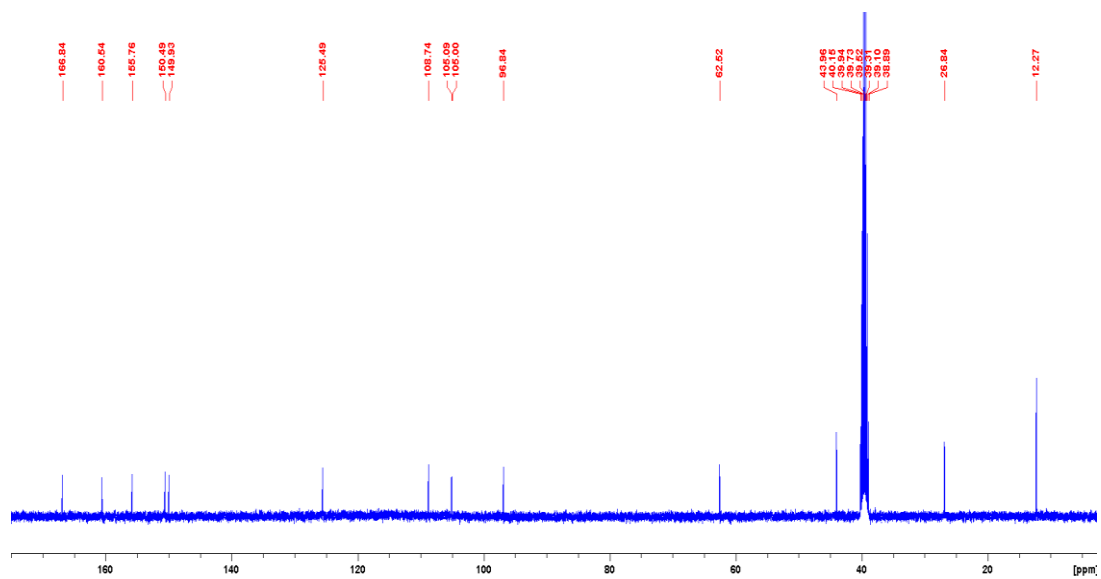
Supporting Figure 2: ¹H-NMR of compound 2.



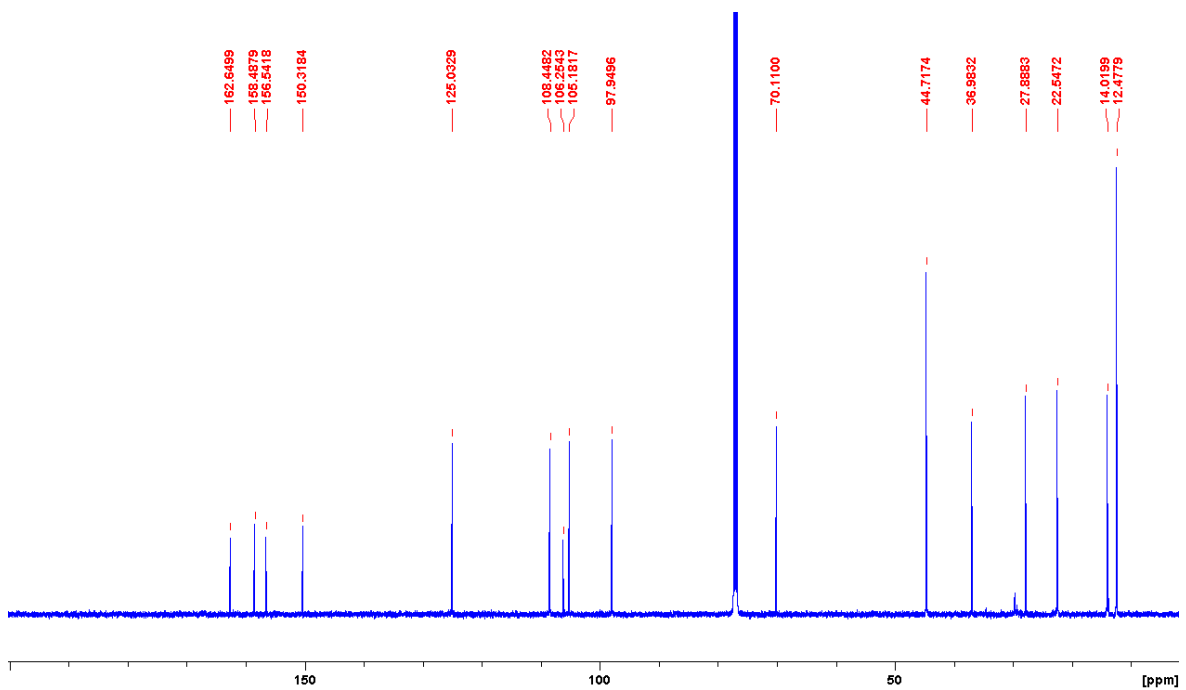
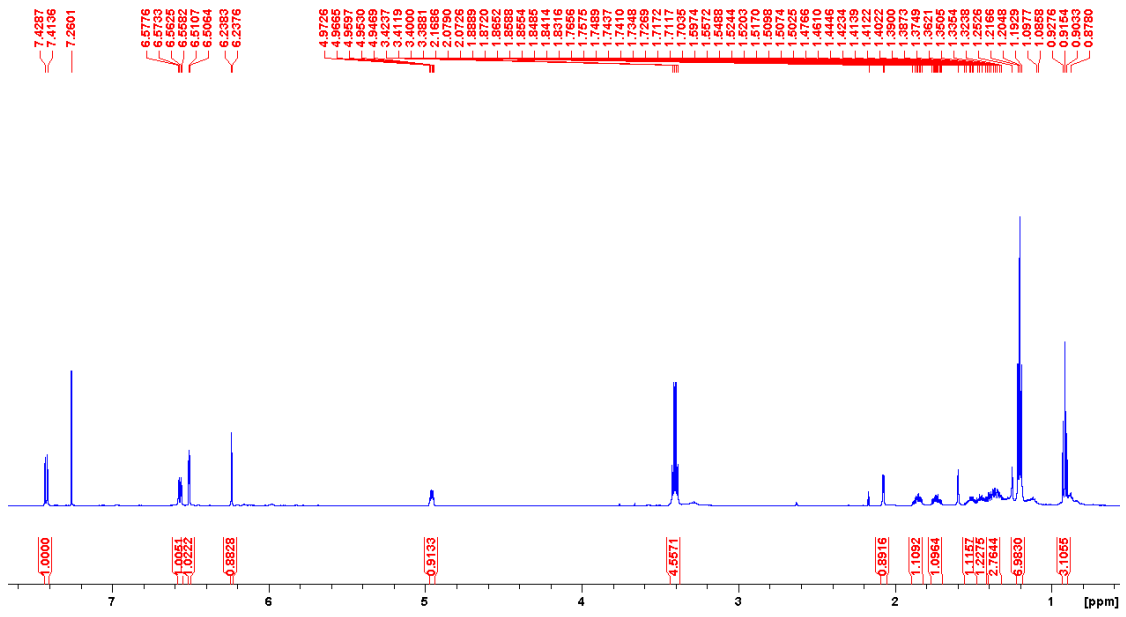
Supporting Figure 3: ¹³C-NMR of compound 2.

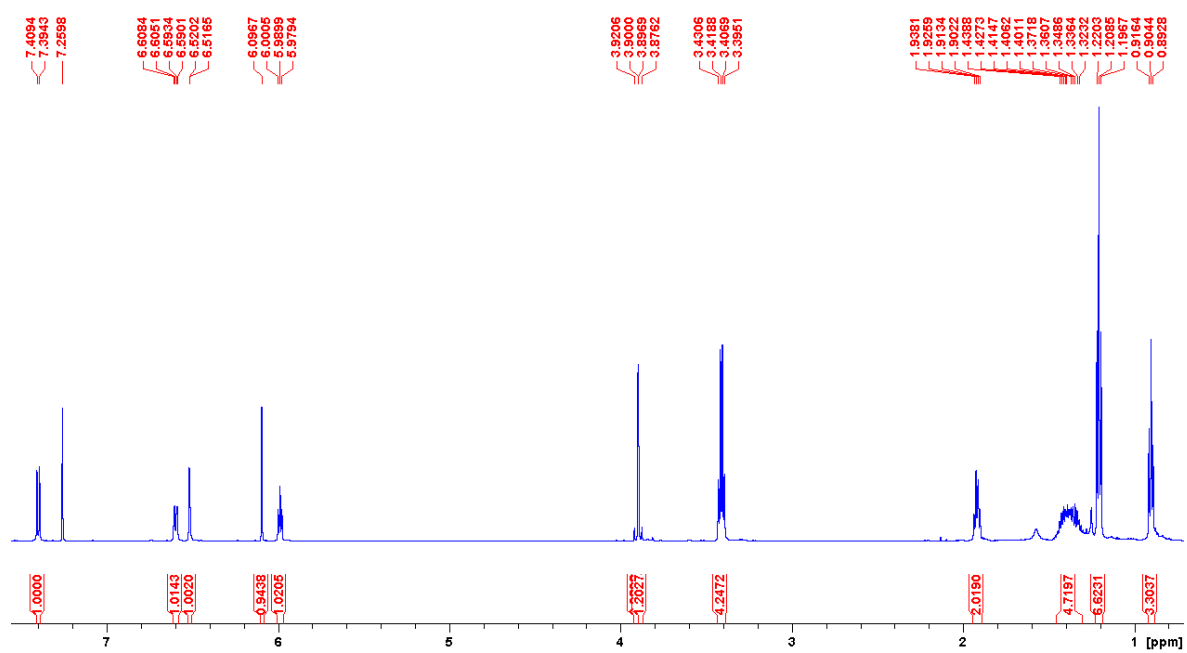


Supporting Figure 4: ^1H -NMR of compound 3.

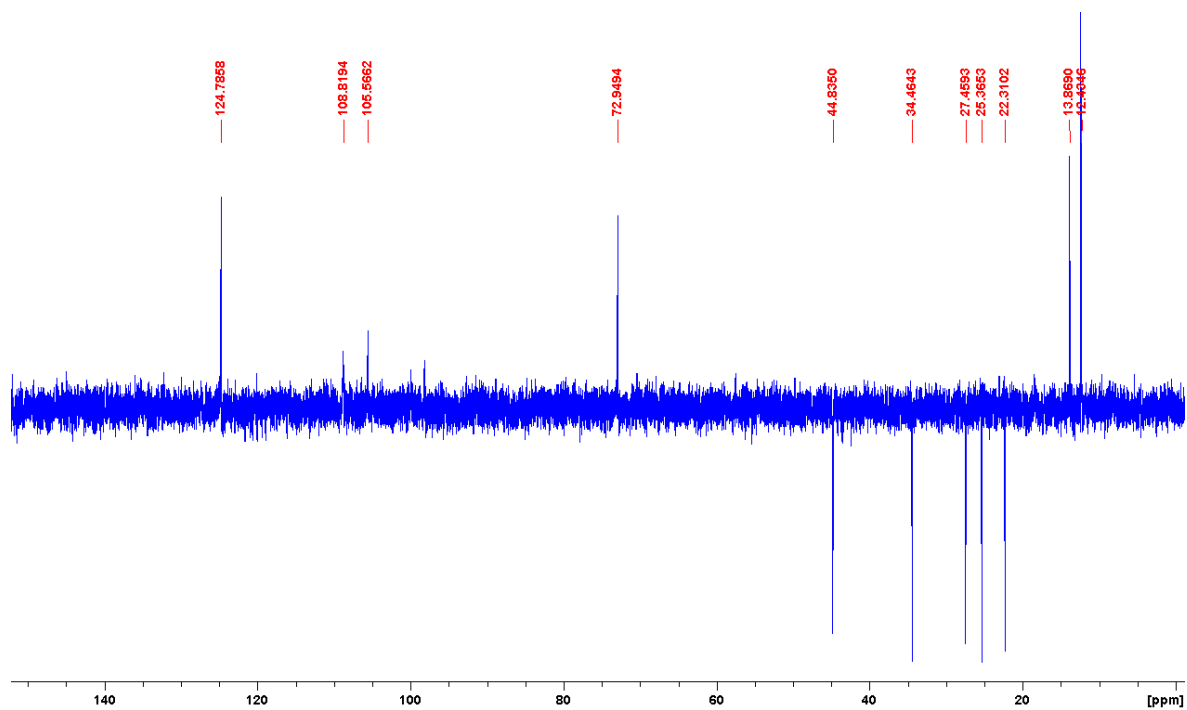


Supporting Figure 5: ^{13}C -NMR of compound 3.





Supporting Figure 8: $^1\text{H-NMR}$ of compound 5.



Supporting Figure 9: $^{13}\text{C-DEPT-NMR}$ of compound 5.

3. Mutagenesis

OmpG/ Δ L6II constructs derived from *E. coli* K-12 *ompG* gene without signal sequence and C-terminal hexa-histidine tag as reported by Grosse *et al.*³ served as template for the introduction of two cysteine residues. On the basis of OmpG/ Δ L6II plasmids C13 and C154 were introduced by site directed mutagenesis using primers 5'-CCGTGTCGAAACGTGTACAGGTCTGCAGTATACCTTCAACG-3' and 5'-CGTTGAAGGTATACTGCAGACCTGTACACGTTTCGACACGG-3'.

Sequencing of the newly constructed primers confirmed introduction of cysteines at the desired positions:

Sequencing Data for OmpG/ Δ L6-M13C-E154C

```
TTAATGGTGGTGGTGGTATGATGGAACGAGTAATTTACGCCGACACCTGCATAATGGAATTT
ATCACTGTCGCCTTCGTCGTGATCCTGCCACTCAAACGCGTATTCCAGCGAAACGGAAAG
TCCGTTCTGGAAATCATAACCGTAAAATAAACCTACACGGTTAAAATCATGGCCGGCGCG
ATCCAGCCCAATGCGCGTATACGGCGTCACCGAGTGGTTGCCGAGCGTCAGCGGCAAATA
GGCGCGAATTTCTTGCCTGGAAAACCTACCGTTATTGCGGCTGTCGTCCATATTGAAGCC
GCGCTCGAGATAATAGTTCACTCGCAAGGCAACCGTTTCGTTGAAGGTATACTGCAGACC
TGTACACGTTTCGACACGGGTATCAGCGTAAACCGTAGTGTTTCAGATCGTTGGCAAATTT
ATACATCGACAACCAACCGTTGAAACGTAAATCGTCAGTCAGTTTTCACATCCCAGTCTGG
CGCGATTTTCCAGCGCTGCATATTCGCCGTGTCTTTACCCGGTTCATCAACGTAGTGATA
ACCATAATTACGAAACCGCCGGTCAGGCCGAAACTGAAATCATCGTTTTTCGAGGAACTG
ATAATGCACCTCCAGCTCCGGGCGATCAAACCACGTTCCACGTTTACCCGCGCTATAATC
TACCGGCCCTTCTGATAATAGGCCAGAGCAATTCTCCACGGCCCGTTGGCGGCATTAAT
ATAGACTGAAGGCTCCGCCAGCCCATCCATATCTTCGCCATAACCCTCGACGTTTTCTAT
TTCGTAACAGGCGCGGATATTAAGTGCCAGTCGTTTCCTTTCCTCCAT
```

Sequence translation was performed by using online tool *ExPASy translator*. Cysteine mutations highlighted in the amino acid sequence:

```
MEERNDWHFN IGACCYEIENV EGYGEDMDGL AEPSVYFNAA NGPWRIALAY YQEGPVDYSA
GKRGTWFD RP ELEVHYQFLE NDDFSFGLTG GFRNYGYHYV DEPGKDTANM QRWKIAPDWD
VKLTDDLRFN GWLSMYKFAN DLNNTGYADT RVETCTGLQY TFNETVALRV NYYLERGFNM
DDSRNNGEFS TQEIRAYLPL TLGNHVSVPY TRIGLDRAGH DFNRVGLFYG YDFQNGLSVS
LEYAFEWQDH DEGDSKDFHY AGVGVNYSFH HHHHH
```

4. Protein methods

Expression and Purification

OmpG mutant was produced as inclusion bodies after transformation in the porin-deficient *E. coli* BL21 (DE3)omp9 strain (B⁻ *ompT hsdSB(rB⁻ mB⁻) gal dcm* (DE3) $\Delta lamB$ *ompF::Tn5* $\Delta ompA$ $\Delta ompC$ $\Delta ompN$:: Ω)⁴ as described by Grosse *et al.*³ Cells were grown in 2YT medium with 100 μ g/mL ampicillin and 1% glucose at 37 °C until OD₅₉₅~0.7. Expression was induced by addition of IPTG (1 mM). Cells were grown for 4 h, harvested by centrifugation (4000 g, 4 °C, 30 min) and resuspended in TN buffer (50 mM Tris/HCl, pH 8.0, 100 mM NaCl). Before cell lysis in an *M-110P homogenizer* (*Microfluidics*), DNase and 1mM PMSF were added. The resulting suspension was centrifuged (22000 g, 4 °C, 25 min) and supernatant discarded. Inclusion bodies were washed in Triton-TN buffer (50 mM Tris/HCl, pH 8.0, 100 mM NaCl, 2.0% (v/v) Triton-X-100) for 3 times and in TN buffer (50 mM Tris/HCl, pH 8.0, 100 mM NaCl) again for 3 times. Washed inclusion bodies were suspended in TN-buffer, aliquoted and stored at -20 °C until further usage.

Refolding

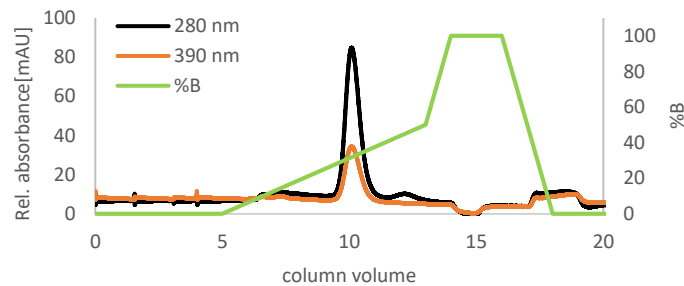
Purified inclusion bodies were resuspended in denaturation buffer (25 mM Tris/HCl, pH 8.0, 6 M urea, 1 mM TCEP), heated for 10 min at 60 °C and centrifuged at 20000 g for 3 min. The supernatant was moved to a fresh tube and the concentration determined. Refolding was performed by rapid dilution in refolding buffer (25 mM Tris/HCl, pH 8.0, 2.5 M urea, 1 mM TCEP, 3% OG) to a concentration of 1 mg/mL OmpG and mildly shaken overnight at 30 °C. Misfolded porin was removed by digestion with proteinase K (10 μ g/mL, 30 min, 25 °C) and the reaction stopped with 13 mM PMSF. Afterwards, refolded protein was concentrated and washed with IEC starting buffer prior to ion exchange chromatography (*HiTrap Q*, 10 mM Tris/HCl, pH 8.0, 10% glycerol, 0.05% LDAO, NaCl gradient, see supporting table 1 and figure 10) Purified OmpG was concentrated to at least 5 mg/mL and stored at -80 °C until further usage.

Supporting Table 1: NaCl gradient of ion exchange chromatography in IEC buffer.

Column volume	% 10 mM NaCl	% 1 M NaCl
0	100	0
6	100	0
16	50	50

Labelling

Refolded OmpG was diluted to a concentration of 3.0 mg/mL in labelling buffer (100 mM KPi , pH 7.5, 0.4% C_8E_4). Coumarin **3/5** was diluted in acetonitrile and added in a tenfold concentration to the protein together with 1 mM TCEP. After an incubation of about 30 minutes at 37 °C, the addition was repeated and the incubation extended overnight. OmpG hybrids were separated from excess coumarin by size exclusion PD-10 columns and another IEC chromatography (see supporting table 1 for gradient, figure 11). Afterwards, the product was concentrated to 5-10 mg/mL and dialyzed in storage buffer (25 mM Tris/HCl, pH 8.0, 10% glycerol, 100 mM NaCl, 0.4% C_8E_4). The final product was stored at -80 °C until further usage.



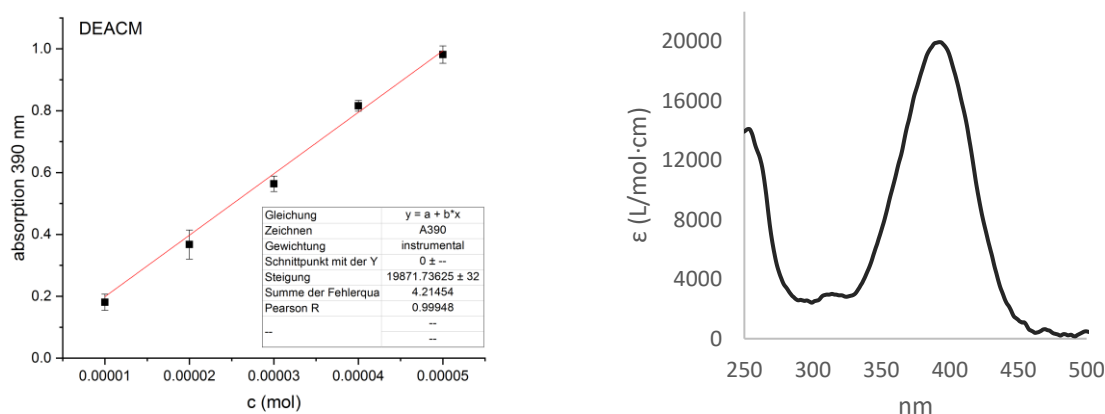
Supporting Figure 10: exemplary FPLC trace of **OmpG-1**. Absorbance at 390 nm (green) provides evidence for a successful labelling.

5. Determination of labelling efficiency by absorption

The labelling efficiency of OmpG hybrids was determined by absorption measurements. Distinct absorption maxima of both OmpG and label were compared in order to determine the amount of label attached to the protein. Therefore, all molar extinction coefficients (ϵ) were determined for a calculation of concentrations. The molar extinction coefficient of OmpG was determined by its amino acid sequence applying the online tool *ExpASY ProtParam* (see supporting table 2). The molar extinction coefficients of the labels were determined by absorption measurements of a concentration series of the small molecule.

OmpG-2

In a first step, the molar extinction coefficient of DEACM was determined at its absorption maximum $\epsilon_{\max}(\lambda) = 390 \text{ nm}$ (see supporting figure 12). For the extinction coefficient of the doubly labelled hybrid the value of DEACM at 280 nm was added twice to the extinction coefficient of unlabelled **OmpG-1** (see supporting table 2).

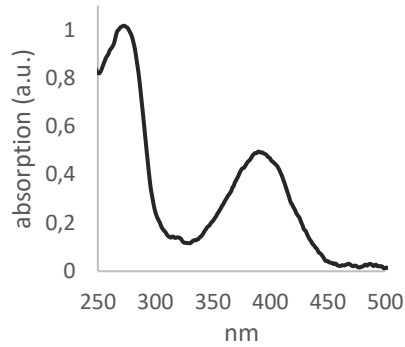


Supporting Figure 11: left – graphical determination of ϵ . Right – absorption spectrum of DEACM.

Supporting Table 2: Summary of extinction coefficients.

	ϵ 280 nm	ϵ 390 nm
	L/mol·cm	L/mol·cm
OmpG-1	71260	0
DEACM	3500	19900
OmpG-2	78260	19900

From the absorption spectrum in Supporting Figure 13 absorption at 280 and 390 nm can be drawn. In Supporting Table 3 the concentration is calculated from absorbance values. DEACM has approximately the double concentration of OmpG which indicates full labelling.



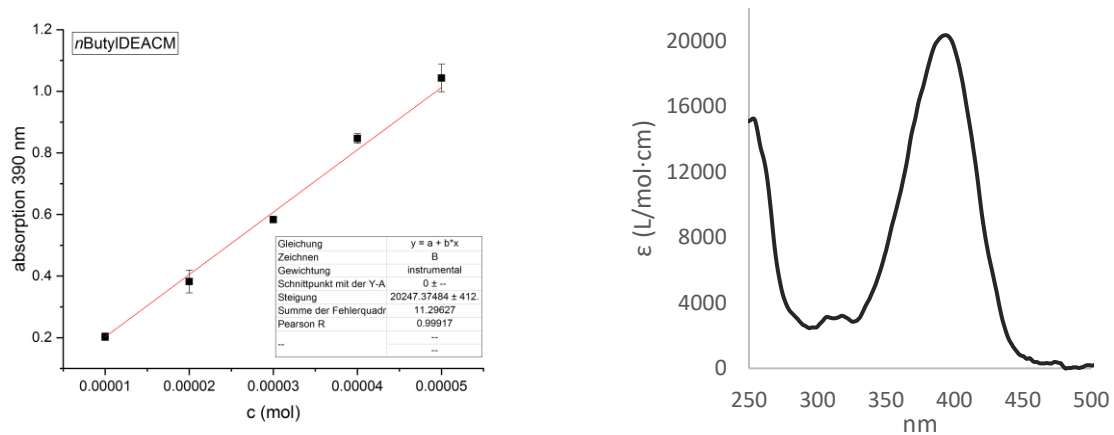
Supporting Figure 12: absorption spectrum of **OmpG-2**.

Supporting Table 3: concentration of **OmpG-2** determined at 280 and 390 nm.

OmpG-2	unit	280 nm	390 nm
ϵ	L/mol·cm	78260	19900
A	a.u.	0.965	0.495
c	μ M	12.3	24.9

OmpG-4

For *n-buty*/DEACM the same procedure was performed as above. The results are shown in Supporting Figure 14 and Table 4.

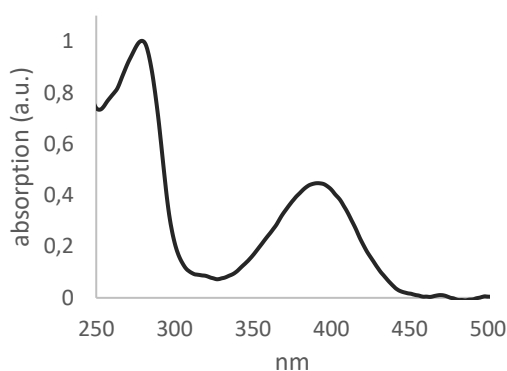


Supporting Figure 13: left – graphical determination of ϵ . Right – absorbance spectrum of *n-buty*/DEACM.

Supporting Table 4: Summary of extinction coefficients.

	ϵ 280 nm	ϵ 390 nm
	L/mol·cm	L/mol·cm
OmpG-1	71260	0
<i>nbutyl</i> DEACM	3500	20200
OmpG-4	78260	20200

From the absorption spectrum in Supporting Figure 15 absorption at 280 and 390 nm can be drawn. In Supporting Table 5 the concentration is calculated from absorbance values at the corresponding wavelengths. Comparing the concentration of *nbutyl*DEACM and OmpG does not confer with the 2:1 ratio which results in a labelling efficiency of 87%. This is probably due to the hydrophobic nature of the label and the lower solubility in aqueous media.



Supporting Figure 14: absorption spectrum of **OmpG-4**.

Supporting Table 5: concentration of **OmpG-2** determined at 280 and 390 nm.

OmpG-4	unit	280 nm	390 nm
ϵ	L/mol·cm	78260	20200
A	a.u.	1.00	0.447
c	μ M	12.8	22.2

6. Mass spectrometry

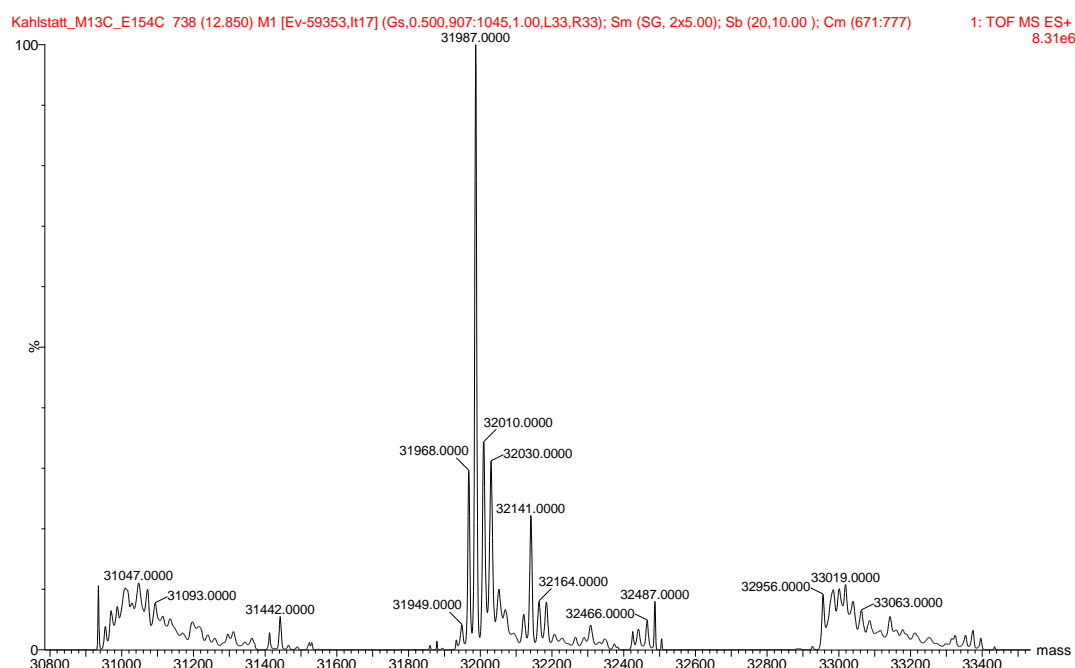
Depending on their concentration and the expected protein masses, 1-10 μL of the buffered protein solutions were desalted online using a *Waters ACQUITY H-Class* HPLC-system equipped with a MassPrep column (*Waters*). Desalted proteins were eluted into the ESI source of a Synapt G2Si mass spectrometer (*Waters*) by the following gradient of buffer A (water/0.05% formic acid) and buffer B (acetonitrile/0.045% formic acid) at a column temperature of 60°C and a flow rate of 0.1 mL/min: Isocratic elution with 5% A for two minutes, followed by a linear gradient to 95% B within 8 min and holding 95% B for additional 4 minutes.

Positive ions within the mass range of 500-5000 m/z were detected. Glu-fibrinopeptide B was measured every 45 s for automatic mass drift correction. Averaged spectra were deconvoluted after baseline subtraction and eventually smoothing using MassLynx instrument software with MaxEnt1 extension.

Molecular weight of **OmpG-1** was calculated from its sequential data by using the online tool *ExpASY ProtParam*.

OmpG-1: OmpG/ Δ L6-M13C-E154C

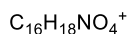
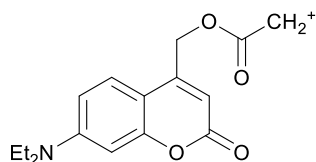
calculated	31986.79 Da
found	31987.00 Da



Supporting Figure 15: mass spectra of **OmpG-1** (OmpG/ Δ L6-M13C-E154C).

OmpG-2: OmpG/ Δ L6-M13C-E154C/2xDEACM

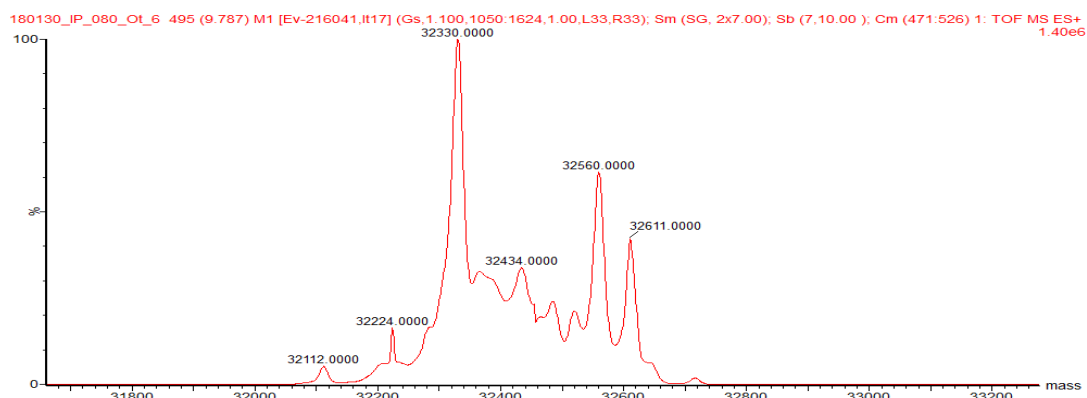
Molecular weight of **OmpG-2** was calculated by addition of two DEACM residues (see below) to the confirmed mass of **OmpG-1**.



Exact Mass: 288.12303

Mol. Wt.: 288.32245

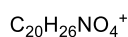
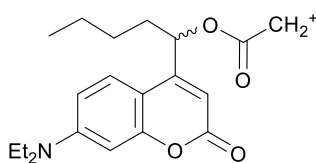
calculated	32563.24 g/mol
found	32560.00 g/mol



Supporting Figure 16: mass spectra of **OmpG-2** (OmpG/ Δ L6-M13C-E154C/2xDEACM).

OmpG-4: OmpG/ Δ L6-M13C-E154C/2xn-butyl/DEACM

Molecular weight of **OmpG-4** was calculated by addition of two *n*-butyl/DEACM residues (see below) to the confirmed mass of **OmpG-1**.

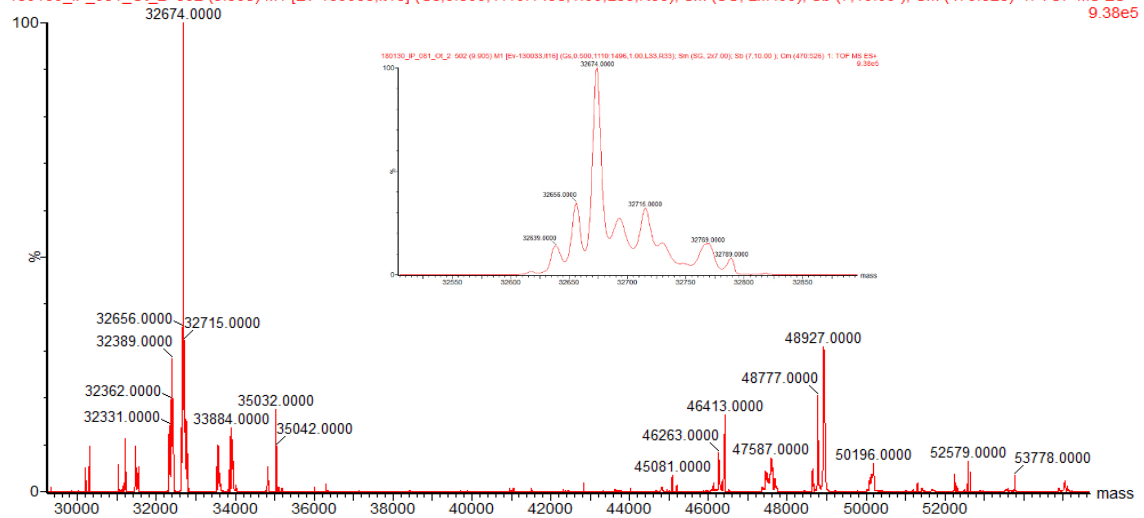


Exact Mass: 344.18563

Mol. Wt.: 344.43045

calculated	32675.37 g/mol
found	32674.00 g/mol

180130_IP_081_Ot_2_502 (9.905) M1 [Ev-130033,It16] (Gs,0.500,1110:1496,1.00,L33,R33); Sm (SG, 2x7.00); Sb (7,10.00); Cm (470:526) 1: TOF MS ES+ 9.38e5



Supporting Figure 17: mass spectra of OmpG-4 (OmpG/ Δ L6-M13C-E154C/2*xn*-butyl/DEACM).

7. Spectroscopy

Absorption spectra

UV-vis absorption spectra were recorded on an *Evolution 300 Spectrometer (Thermo Scientific)* using a 70 μL cuvette with 1 cm path length by *Hellma*.

Fluorescence spectra

Fluorescence spectra were recorded on a *Tecan Plate Reader infinite M200 PRO*. 50 μL of sample solution were pipetted into a black 96 well plate by *Corning*. Samples were excited at 390 nm, emission detected between 420 and 650 nm.

Irradiation

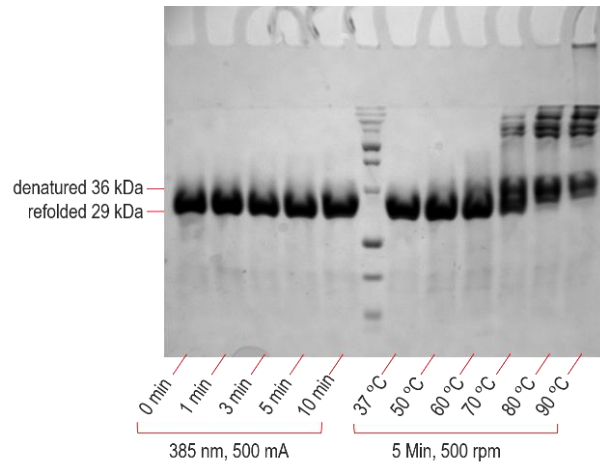
During Black Lipid Membrane measurements OmpG hybrids were irradiated by a *Thorlabs* fiber-coupled LED M385F1 equipped with a *Thorlabs* LED driver DC2100 at 700 mA, 95 mW for 3-10 min (1000 μm fiber).

Samples for SDS PAGE were irradiated with a custom made Multi-LED device equipped with LEDs by *Roithner Lasertechnik* at 385 nm for different time intervals (UVLED-385-310-SMD, 385 nm, 310 mW, 500 mA).

Read out of SDS PAGE fluorescent bands was performed on a *transilluminator BIO View UV light (USDT-30ML-8R, Biostep)* with excitation at 365 nm (5 mW).

Stability test

In order to proof stability of OmpG against irradiation and possible heating during irradiation a stability test was performed with unmodified **OmpG-1** (see supporting figure 18). In an SDS PAGE a read out was done comparing the different migration behavior of native and denatured OmpG. On the left side samples of **OmpG-1** were irradiated for 0, 1, 3, 5 and 10 min at 385 nm (details see above). On the right side samples were heated for 5 min at 37, 50, 60, 70, 80 and 90 °C. OmpG appears stable against irradiation at 385 nm for 10 min and heating up to 60 °C.



Supporting Figure 18: SDS PAGE of **OmpG-1**. Stability test against - left side: irradiation; - right side: temperature.

8. Black Lipid Membrane Measurements

OmpG conductances were electrophysiologically monitored by the Black Lipid Membrane (BLM) method. Experiments were performed in 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC, Avanti polar lipids) as previously described.³ *N*-decane-solubilised lipid (25 mg/mL) was painted over a 200 μm circular hole, separating two chamber compartments (polystyrene cuvette: CP2A, bilayer chamber: BCH-22A, *Warner Instruments*) filled equally with BLM buffer (10 mM Tris/HCl, pH 7.4, 1 M NaCl). The measurements were carried out under red light. Detergent refolded and purified porins (7.5–15 μg) were added to one compartment next to the planar lipid layer and a voltage-gradient was imposed across the membrane whilst waiting for insertion of single channels. When a single channel was detected, the voltage was changed between + 60 mV and – 60 mV and the current recorded. The voltage was then set to 40 mV and the sample was exposed to blue light (ThorLabs DC2100, with LED M365L2: 365 nm) for up to 3 minutes. After that, voltage was changed as before and the currents recorded again.

Electric current was recorded using a Multipatch 700B patch-clamp amplifier connected to a Digidata 1440A A/D converter.

Current traces were analyzed using the *Clampex* 10.4 software (*Axon Instruments*), from a data collection frequency of 5 kHz and a sampling rate of 200 Hz. U-I-plots of five measurements were used to determine the conductivity.

9. Supporting References

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