Flavin-induced charge separation in transmembrane model peptides

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Supporting Information

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1. Materials and methods

All chemicals were purchased from Sigma-Aldrich, Merck, ABCR, TCI or Alfa Aesar. If not mentioned otherwise, the chemicals were used as received. Used solvents had at least quality level *pro analysi*. Dry solvents for synthesis were purchased from Acros Organics and stored under argon. For HPLC (high performance liquid chromatography) purification used organic solvents (Biosolve or Acros Organics) were HPLC grade and ultrapure water was used (prepared by using a Millipore-Q8 system, Merck).

For peptide synthesis, fluorenylmethoxycarbonyl (=Fmoc)-protected amino acids and the used coupling reagents (listed below) were purchased from Iris Biotech or Novabiochem. UV-grade chloroform and methanol for circular dichroism (CD) and oriented circular dichroism (OCD) sample preparation were obtained from VWR International. The lipid (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, POPC) was purchased from Avanti Polar Lipids and used without further purification.

The high-resolution mass spectra for synthetic compounds were recorded at a Finnigan MAT95 and either probed using EI (electron impact ionization) or FAB (fast atom bombardement).

UV-Vis absorption measurements were performed on a Lambda 750 UV/Vis spectrometer (Perkin Elmer) equipped with a PTP-6+6 peltier system (Perkin Elmer) and a Corio^{TM} CD thermostat from Julabo. The used parameters were:_spectral bandwidth: 2.0 nm, average time: 0.10 s, data interval: 1.0 nm, lamp change over 319.2 nm and 20°C.

Fluorescence spectra were recorded on a Fluoromax-4 (Jobin Yvon - HORIBA) equipped with a Haake AC200 thermostat from Thermo Scientific. The spectra were recorded using FluorEssence v3.5 software and baseline corrected against Raman backscattering. The used parameters were: increment: 1.00 nm, increment time: 0.2 s, integration time: 0.10 s, slits: 4 nm (or less) and 20°C.

Fluorescence quantum yield measurements were performed on a Quantaurus QY C11347 instrument (Hamamatsu).

Transient absorption spectra were acquired using a home-built system by splitting an 800 nm beam (Astrella, Coherent, 1 kHz 35 fs, 7 µJ) into pump and probe pulses. The pump pulses (400 nm, 2 µJ) were generated by frequency doubling via BBO-crystal. Excitation energy was controlled by aligning the BBO-angle. A modification of the setup allowed the generation of the third harmonic (267 nm) by sum frequency mixing of the 400 nm and 800 nm pulses. Before passing the sample, every second pulse was blocked via an optical chopper (Thorlabs) Probe pulses (white light continuum, 350 – 750 nm) were generated by irradiating a CaF_2 crystal after passing a computer-controlled delay stage (Thorlabs). A small fraction of the with light beam was directed into a reference camera (linescan Series2000, 512 pixels, Si detector, Entwicklungsbüro Stresing), to acquire a better signal noise ratio. Pump and probe pulses were overlapped in a cuvette (Starna cuvette, suprasil quartz, optical path length of 1 mm, continuously stirred using a miniaturized magnetic bar). After passing the sample, the white light was recorded by the main camera (same type as above). Data were processed with an in-house written LabView program for data recording and correction of the group velocity mismatch. A Matlab program was used to retrieve decay-associated difference spectra (DADS) by convoluting the sum of two exponentials with the instrument response function.

CD spectra were recorded on a J-815 spectropolarimeter (Jasco). Measurements were performed in 1 mm quartz glass cells (Suprasil) in a range of 260 to 185 nm at 0.1 nm intervals. Spectra were recorded at 25°C using a water-thermostated rectangular cell holder. The average of three accumulations at a scan-rate of 10 nm/min, 8 s response time and 1 nm bandwidth were corrected for the baseline of the corresponding peptide-free sample. Afterwards, the CD data were processed with a smoothing method (see Jasco Spectra Analysis software). To finalize, the spectra got calculated to mean residue ellipticities by the known concentration of each peptide.

OCD experiments were performed using an OCD-cell built in the Ulrich group, which is computer controlled and can be integrated in a J-810 spectropolarimeter (Jasco). [3] The OCD spectra were recorded every 45° of rotation of the cell at eight different angles at 25°C to provide a minimization of spectral artefacts.[3] Each spectra got referenced by subtracting the background signal which includes a sample only containing the used lipid.

To prepare the isotropic (in solution) CD samples, each peptide was dissolved with a final concentration of 0.1 mg/mL in MeCN/H_2O. Proteoliposomes (large unilamellar vesicles, LUV)

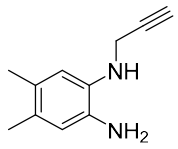
were prepared by cosolubilisation of POPC and the EGFR TM-derived peptides in a $CHCl_3$/MeOH (1:1) mixture with a peptide-to-lipid ratio of 1:50 (mol:mol). After removing the solvents under a gentle stream of nitrogen the samples were further dried for at least 3 h under reduced pressure. Subsequently, the lipid/peptide cake was hydrated by dispersing in 250 µL PBS buffer (phosphate buffered saline, pH 7.2), yielding a peptide at a final concentration of 0.04 mM. The dispersions got homogenized by 10 freeze-thaw cycles to hydrate and to form stable suspensions of multilamellar vesicles. Unilamellar vesicles were formed by hand extrusion (Mini-Extruder, Avanti Polar Lipids) over a polycarbonate membrane with pores of 0.1 µm diameter (Avanti Polar Lipids).

For OCD measurements, the oriented samples of $EGFR_{wt}$-$EGFR_{Fl}6$ in POPC bilayers with P/L ratios of 1:50 (mol:mol) were prepared from solutions in $CHCl_3$/MeOH. A 70 µL aliquot of each sample containing a maximum of 0.1 mg lipid was deposited on a quartz glass plate with a 20 mm diameter. The $CHCl_3$/MeOH solution was allowed to dry in a gentle stream of air until the sample appeared completely dry. Afterwards, the samples were re-hydrated overnight (at least 8 h) at 30°C in the OCD sample cell. The saturated K_2SO_4 salt solution (300–500 mL) at the bottom of the cell maintained a humidity of about ~98%.

2. Synthetic procedures

All compounds and solvents were used without any further purification unless mentioned otherwise. Reaction progress was controlled by thin layer chromatography (TLC) using silica gel aluminium plates (Merck, silica gel 60, thickness 0.2 mm, F_{254} or for reversed phase analysis Merck, silica gel 60 RP-18, thickness 0.2 mm, F_{254S}). Analysis was performed by irradiation with UV light (λ_{exc} = 254 nm or 366 nm) or by staining with ninhydrin solution. The crude reaction mixtures got purified by flash column chromatography on silica gel 60 (Merck, 230-400 mesh) or by automated column chromatography at a IntelliFlash 310 (Varian) using Biotage SNAP Cartridge KP-C18-HS (60g) reversed phase columns. Each compound got analyzed by NMR spectroscopy at a Bruker Avance 500 spectrometer using the Bruker Ascend 500 spectrometer hardware. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (126 MHz) measurements were performed either in DMSO-d_6 or $CDCl_3$ and the resulting spectra were calibrated respective to the solvent signal (δ = 2.50 ppm (^{1}H) and δ = 39.52 ppm (^{13}C) for DMSO-d_6 or 7.260 ppm (^{1}H) and δ = 77.160 ppm (^{13}C) for $CDCl_3$). The spectra were reported in part per million (ppm). Coupling constants (J) are given in Hertz (Hz) and the multiplicity of the signals is given as followed: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, dt (doublet of triplets), td (triplet of doublets).

Compound 2



$C_{11}H_{14}N_2$
Exakte Masse: 174,12

The synthetic route for compound **2** is derived from the literature.[4]

2.50 g 4,5-dimethylbenzene-1,2-diamine (0.018 mol, 1.00 equiv.) and 2.79 g K_2CO_3 (0.018 mol, 1.10 equiv.) were added to 100 mL tetrahydrofurane. Afterwards 1.97 mL 3-brompropine (0.018 mol, 1.00 equiv.) were added dropwise to the reaction mixture. After stirring for 24 h about 100 mL saturated sodium chloride solution were added. And the mixture was extracted three times with ethyl acetate. After drying over sodium sulfate and removing the solvent under reduced pressure, the crude product got purified by silica gel column chromatography using ethyl acetate (17-33 %) in hexane. The product was isolated in 44 % yield as red-brown oil (1.41 g, 0.008 mol).

TLC (Hexane, 16 % EtOAc): R_f = 0.22.

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 2,17 (s, 3H, C-C*H*$_3$); 2.22(s, 3H, C-C*H*$_3$); 2.28 (t, J^4 = 2.33 Hz, ≡C*H*); 3.38 (s, 2H, N*H*$_2$); 3.91 (d, 2H, J^4 = 2.40 Hz, -C*H*$_2$-); 6.56 (s, 1H, aryl-*H*); 6.59 (s, 1H, aryl-*H*).

^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) = 18.97 (*C*H$_3$); 19.34 (*C*H$_3$); 34.37 (-*C*H$_2$-), 71.52 (≡*C*H); 81.66 (CH$_2$-*C*≡CH); 115.45 (aryl-*C*); 118.55 (aryl-*C*); 127.89 (*C*H$_3$-); 133.04 (*C*-N); 133.90 (*C*-N).

HRMS-FAB (m/z): calcd for $C_{11}H_{14}N_2$ [M^+]: 174.1157; found [M^+]: 174.1158.

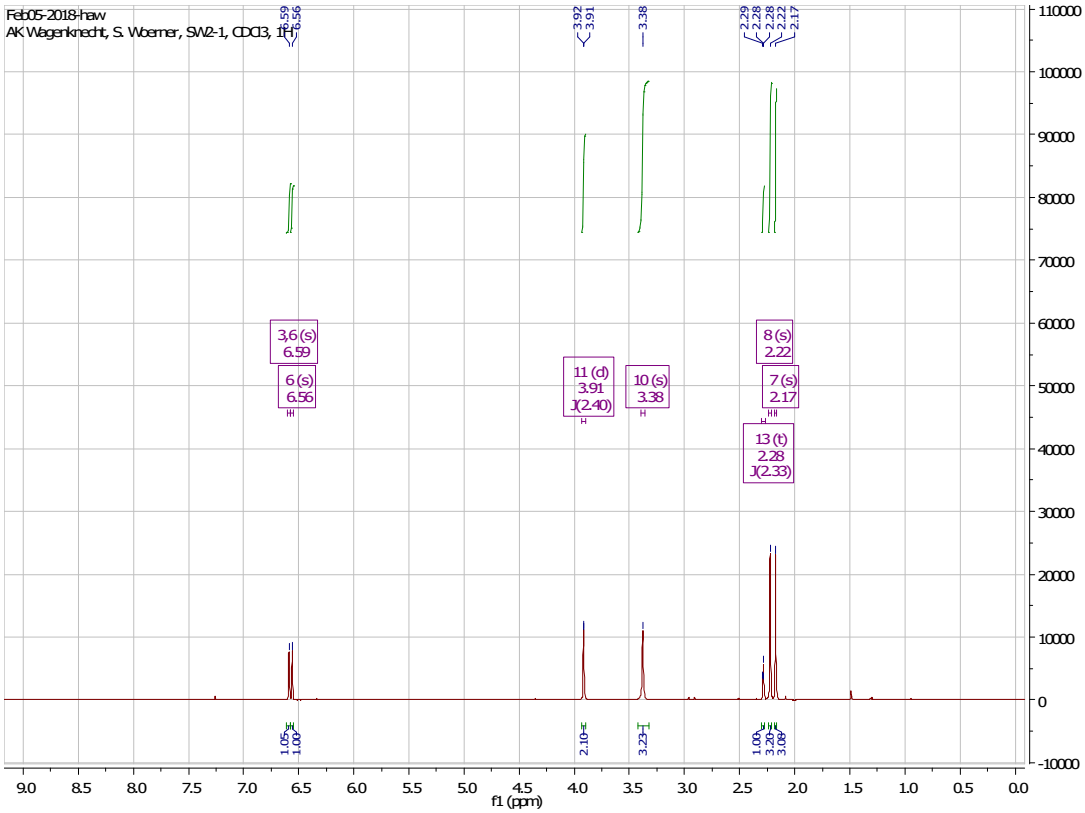


Figure S1: ^{1}H NMR (500 MHz, $CDCl_3$) of **2**.

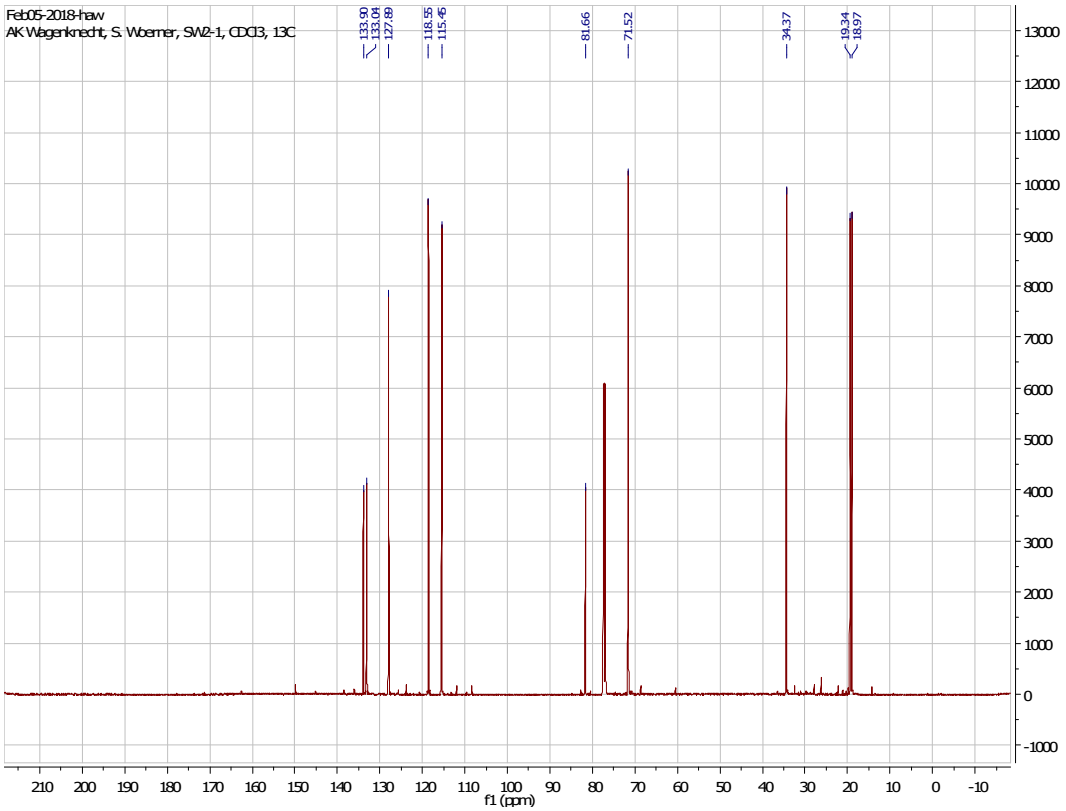
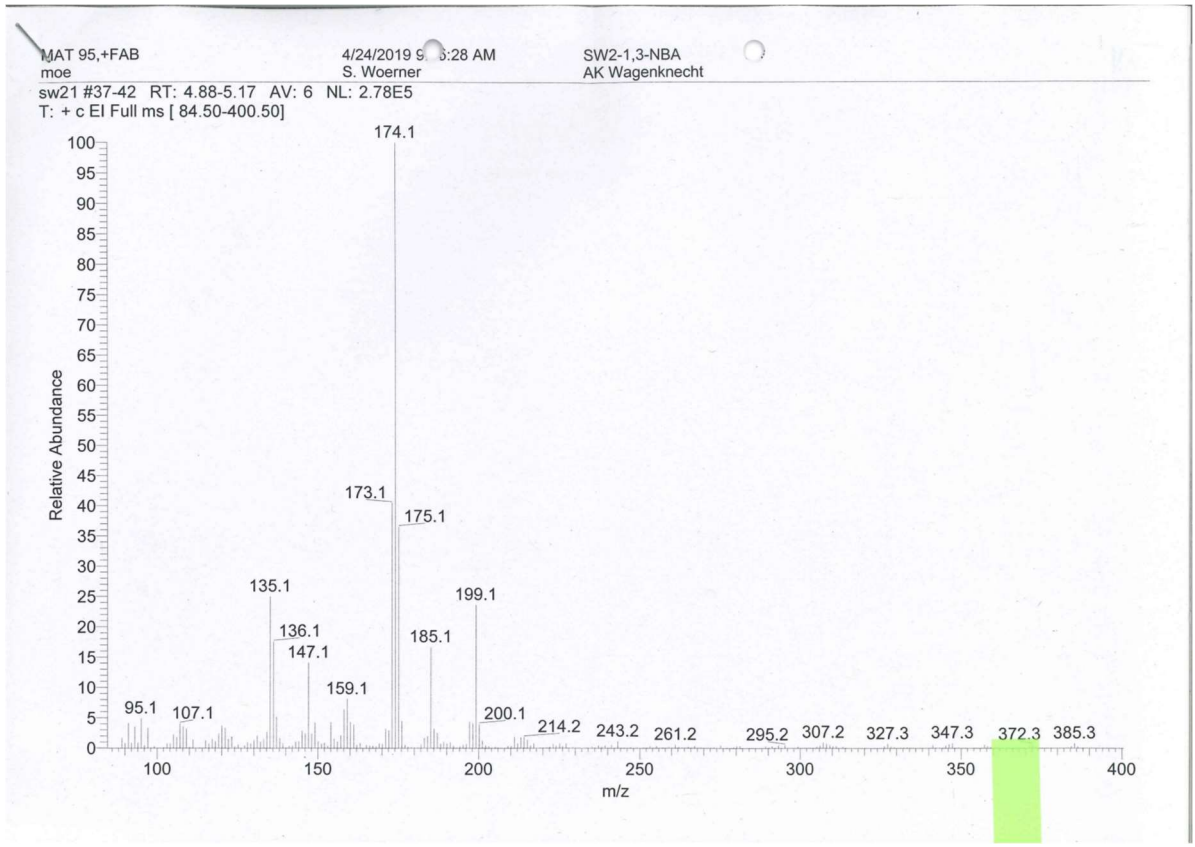


Figure S2: ^{13}C NMR (126 MHz, $CDCl_3$) of **2**.



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m/z= 174.0931-174.1468

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
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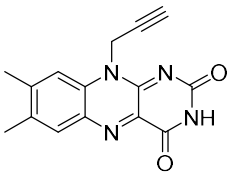
Figure S3: HRMS-FAB of **2.**

Compound 3



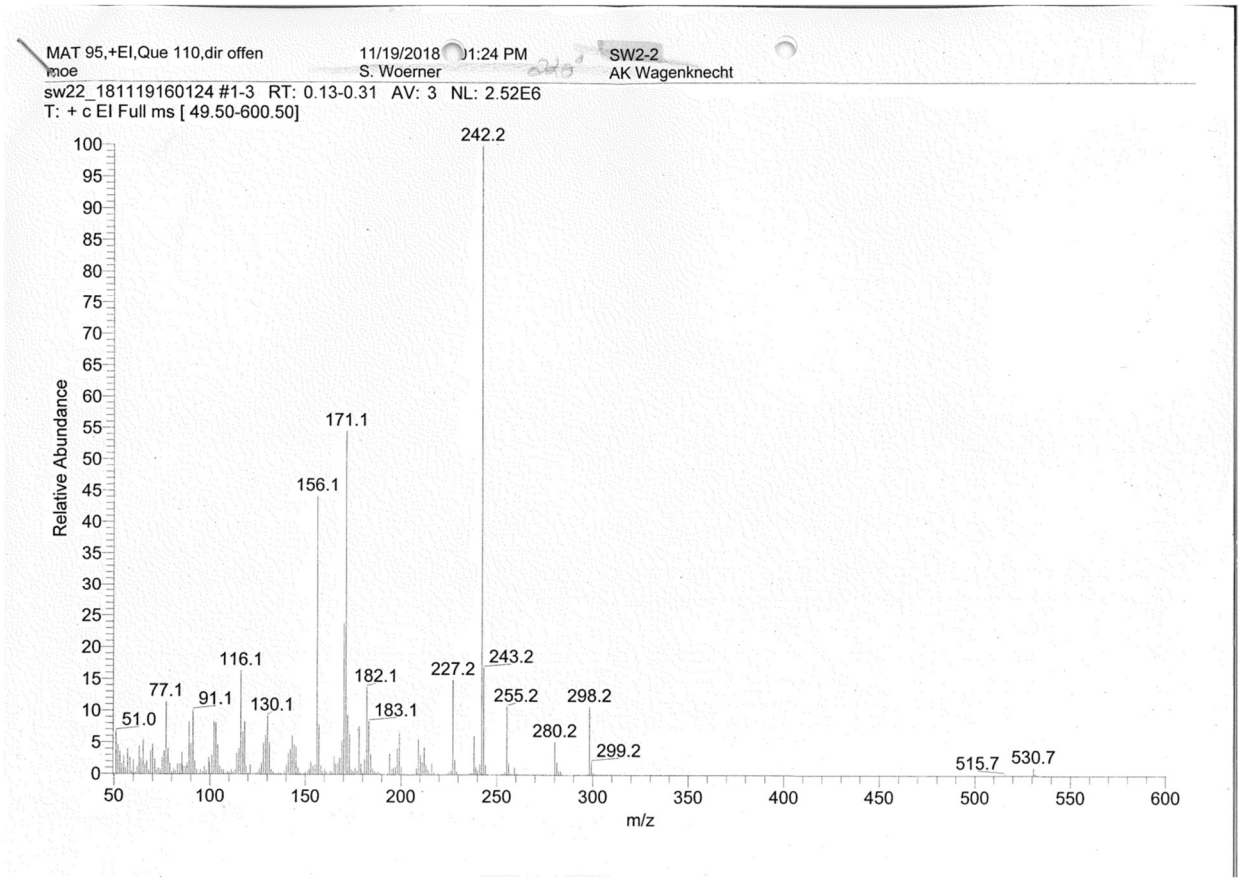
$C_{15}H_{12}N_4O_2$
Exakte Masse: 280,10

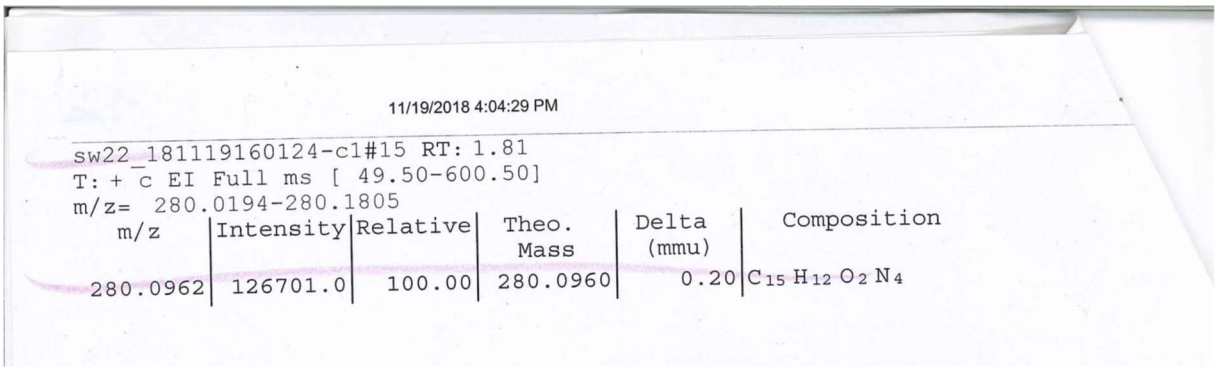
1.41 g **2** (0.0081 mol, 1.00 equiv.), 1.62 g alloxan monohydrate (0.010 mol, 1.25 equiv.) and 1.13 g boron trioxide (0.016 mol, 2.00 equiv.) were combined in 50 mL acetic acid (96%) and stirred over night at 60°C. The solid product got filtered and kindly washed with water. After drying the product was obtained as yellow solid in 79% yield (1.79 g, 0.006 mol).

RP-TLC (H_2O, 33 % MeCN): R_f = 0.14.

Due the bad solubility of compound **3** no significant NMR spectrum could be received.

HRMS-EI (m/z): calcd for $C_{15}H_{12}N_4O_2$ [M^+]: 280.0960; found [M^+]: 280.0962.





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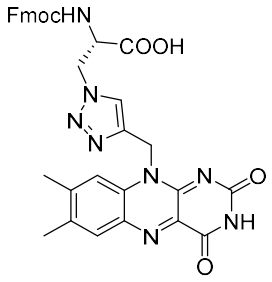
T: + c EI Full ms [49.50-600.50]
m/z= 280.0194-280.1805

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
280.0962	126701.0	100.00	280.0960	0.20	$C_{15}H_{12}O_2N_4$

Figure S4: HRMS-EI of 3.

Compound 1



$C_{33}H_{28}N_8O_6$
Exakte Masse: 632,21

To a suspension of 0.50 g **3** (0.0018 mol, 1.00 equiv.) in 30 mL dry *N*,N-dimethylformamide (DMF) 0.106 g sodium ascorbate (0.00053 mol, 0.30 equiv.), 1.70 g Tris((1-benzyl-4-triazolyl)methyl)amine (0.003 mol, 0.60 equiv.) and 0.199 g $[Cu(CH_3CN)_4]PF_6$ (0.00054 mol, 0.30 equiv.) were added under argon atmosphere. 0.629 g Fmoc-protected β-azido-L-alanine (0.0018 mol, 1.00 equiv.) dissolved in 2 mL dry DMF were added dropwise and the reaction mixture was stirred over night at room temperature. Afterwards, the reaction mixture was evaporated under reduced pressure and the crude product purified by reversed phase C18-silica gel column chromatography using acetonitrile 30-60% in water. Product **1** got isolated as yellow, fluorescent solid (0.78 g, 0.0012 mol, 70 %).

RP-TLC (H_2O, 33 % MeCN): R_f = 0.37.

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 2.30 (s, 3H, Flavin-Me); 2.40 (s, 3H, Flavin-Me) 4.13 (m, 3H, Fmoc-C*H*-CH_2); 4.49 (m, 1H, α-C*H*); 4.55 (m, 1H, β-C*H*); 4.73 (dd, 1H, J^3 = 4.29 Hz, J^2 = 13.78 Hz, β-C*H*); 5.87 (s, 2H, -CH_2-N); 7.30 (m, 2H, Fmoc-*H*); 7.33 (m, 2H, Fmoc-*H*); 7.38 (m, 2H, Fmoc-*H*); 7.59 (d, 2H, J^3 = 7.49 Hz); 7.85 (m, 5H, aryl-C*H*, aryl-C*H*, C*H*-N, N*H*CO; Fmoc-*H*); 11.40 (bs, 1H, Fmoc-N*H*).

^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) = 18.8 (Flavin-Me-*C*); 20.8 (Flavin-Me-*C*); 39.4 (-*C*H_2-); 46.1 (Fmoc-*C*H); 49.6 (β-*C*); 54.0 (α-*C*); 65.8 (Fmoc-*C*H); 116.6 (aryl-C); 120.1 (*C*H-N); 125.0 (Fmoc-*C*);127.1 (Fmoc-*C*); 127.7 (Fmoc-*C*); 128.0 (Fmoc-*C*); 130.5 (aryl-*C*); 131.0 (quart-*C*); 133.7 (quart-*C*); 135.7 (quart-*C*); 135.9 (quart-*C*); 137.3 (quart-*C*); 140.6 (quart-*C*); 140.9 (quart-

C); 143.6 (quart-*C*); 143.7 (quart-*C*); 146.4 (quart-*C*); 150.1 (quart-*C*); 155.6 (quart-*C*); 155.8 (quart-*C*); 159.9 (quart-*C*); 170.7 (quart-*C*).

HRMS-FAB (m/z): calcd for $C_{33}H_{29}N_8O_6$ [M+H$^+$]: 633.2210; found [M+H$^+$]: 633.2209.

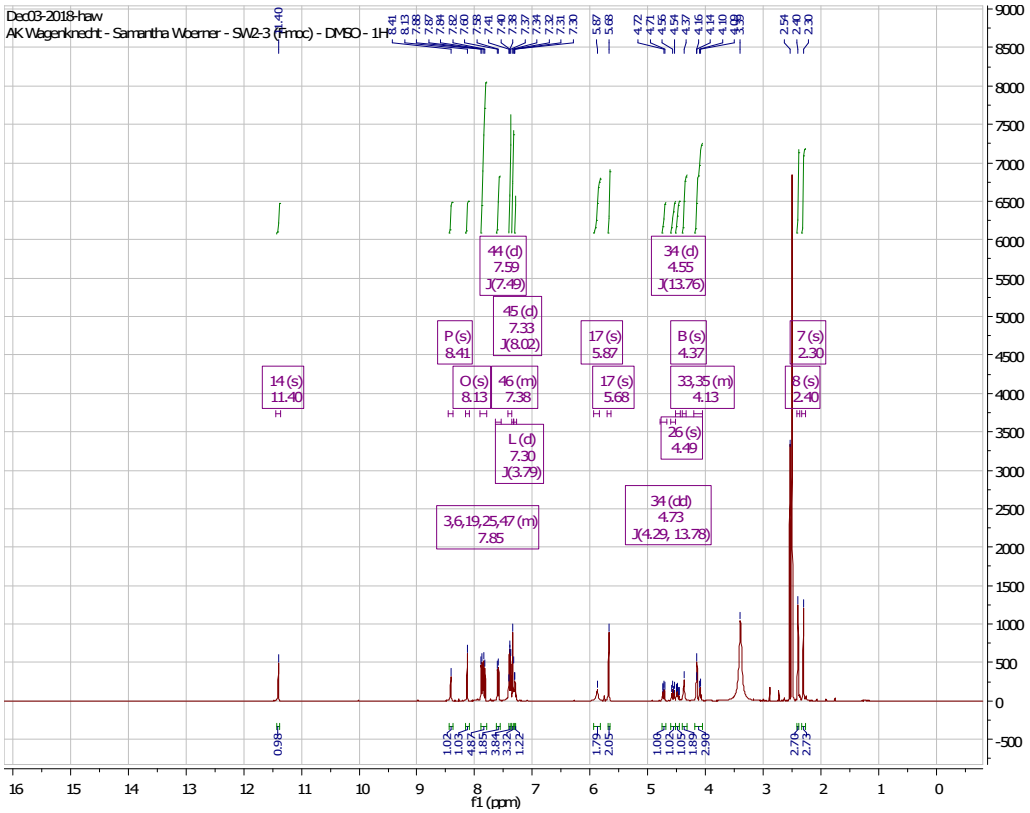


Figure S5. ^{1}H NMR (500 MHz, DMSO-d$_6$) of **1**.

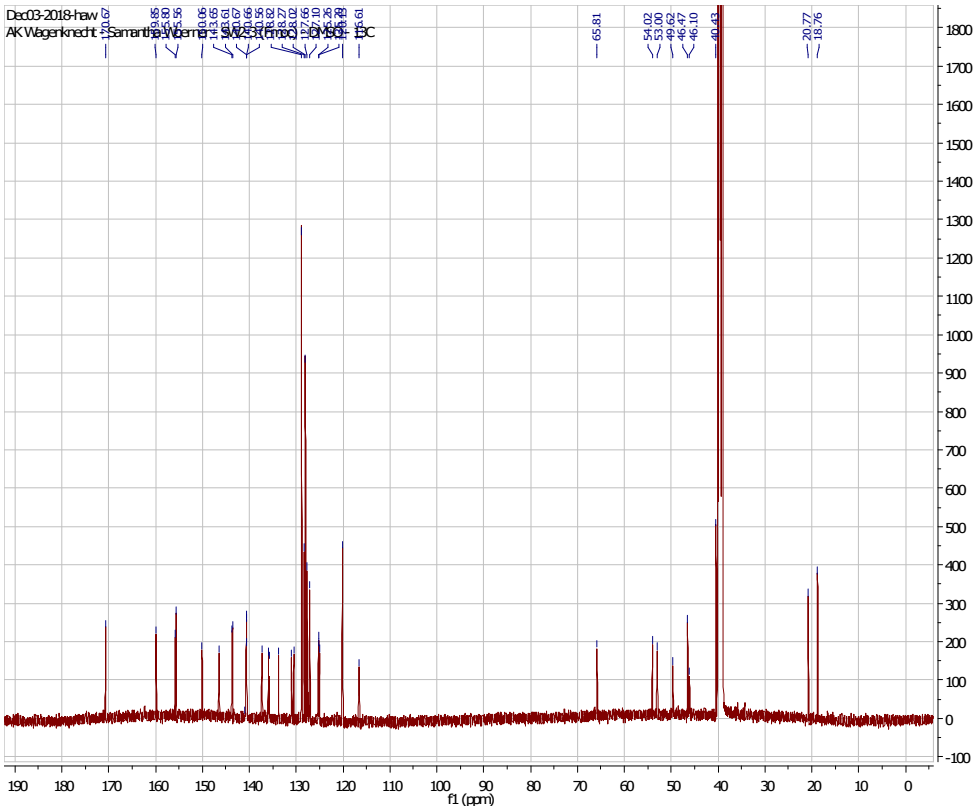
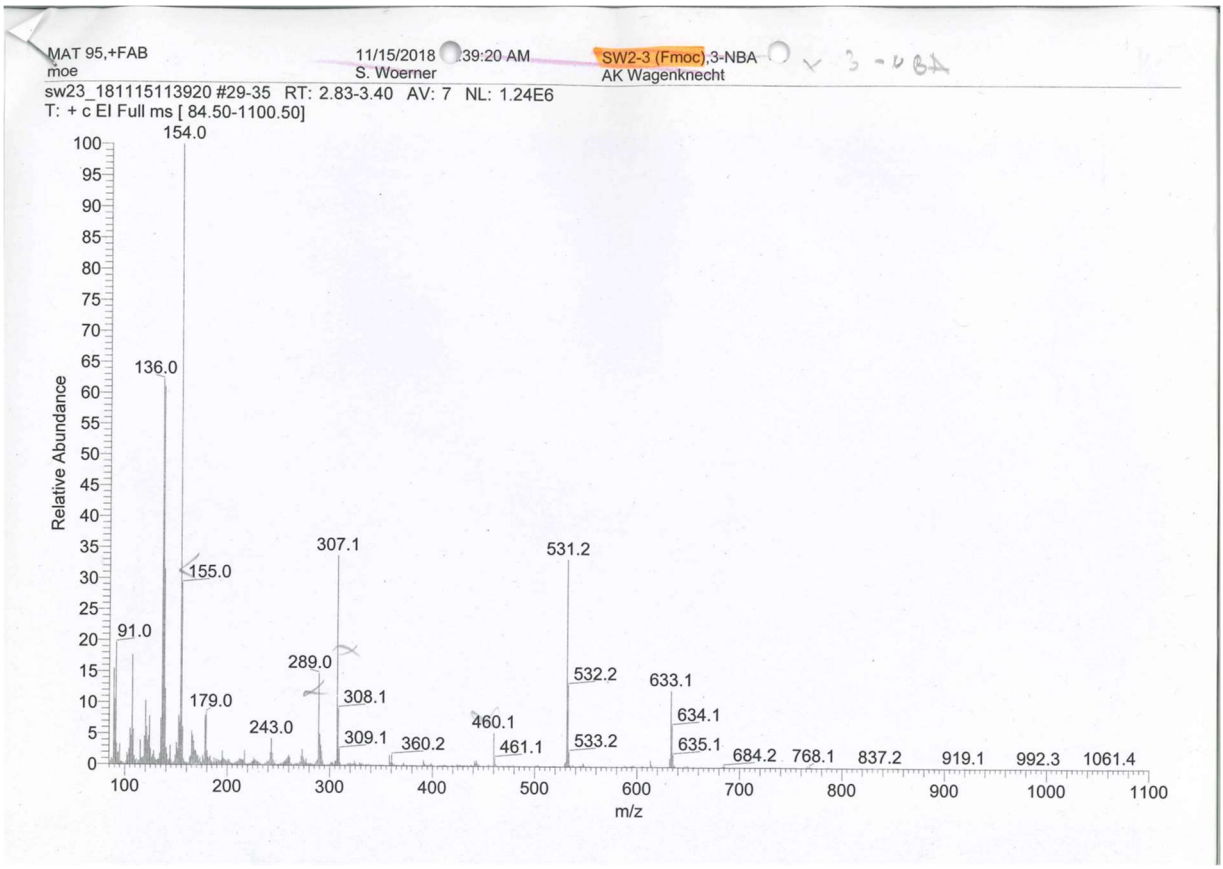
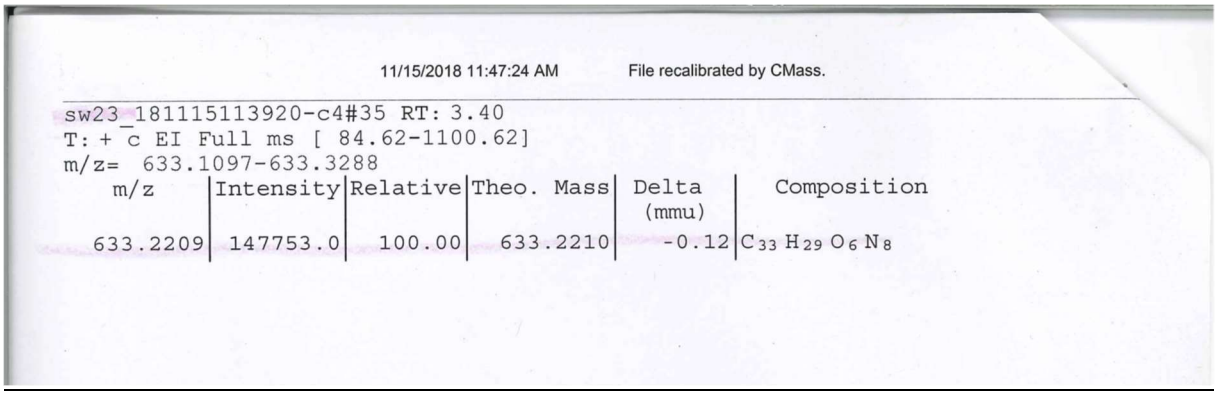


Figure S6: ^{13}C NMR (126 MHz, DMSO-d_6) of **1**.





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m/z= 633.1097-633.3288

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
633.2209	147753.0	100.00	633.2210	-0.12	$C_{33}H_{29}O_6N_8$

Figure S7: HRMS-FAB of 1.

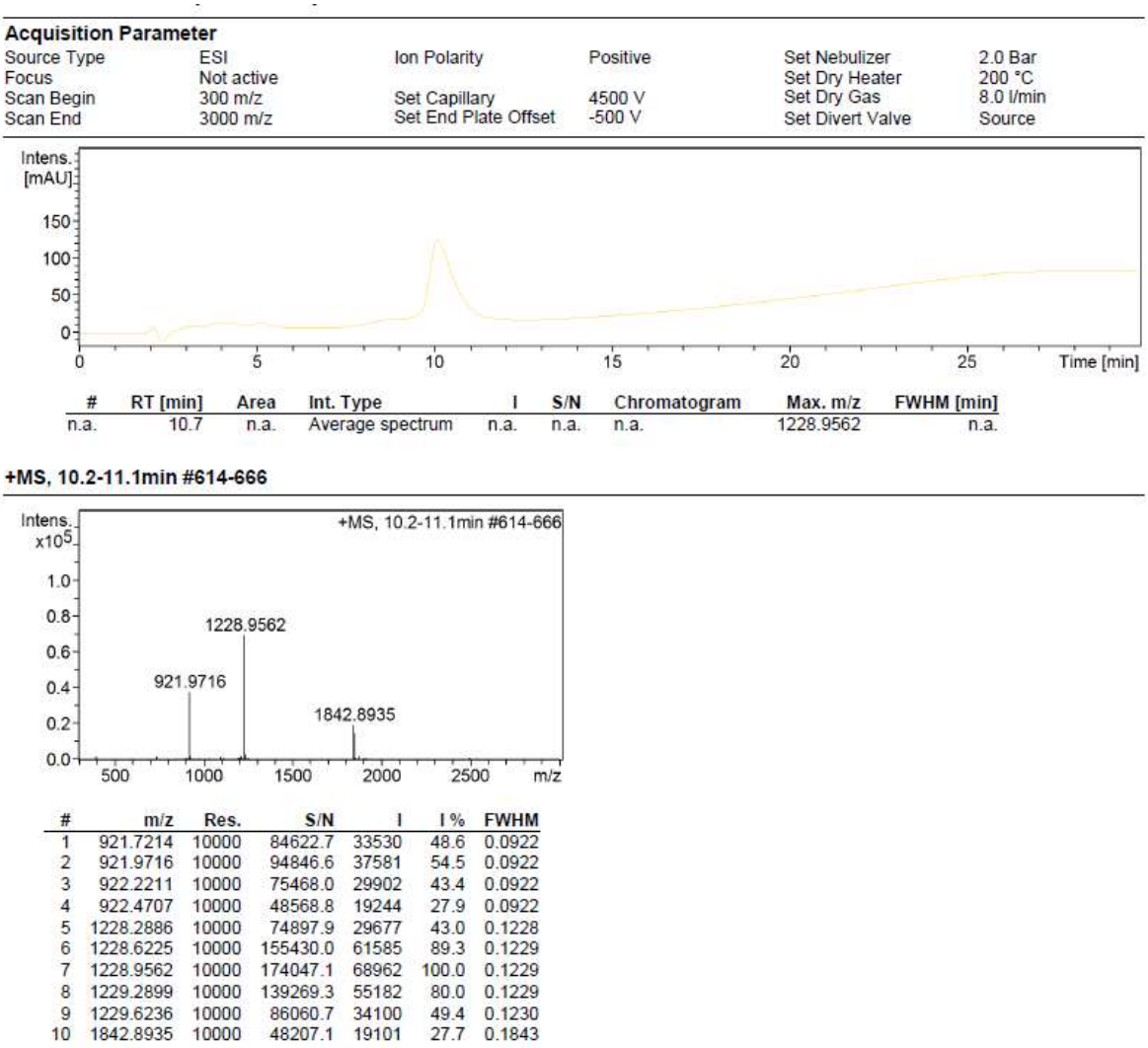
3. Peptide synthesis

Standard Fmoc-solid phase synthesis protocols were used[1] and an automated Liberty blue microwave peptide synthesizer (CEM) was employed. The EGFR TM-derived peptides were synthesized from the C-terminus containing the 33 amino acids. As resin Fmoc-L-Valin-Wang (LL, 100-200 mesh) in an amount of 50 µmol was used. Building block **1** was coupled manually using the standard coupling strategy (1.20 equiv. HOBt*H_2O (1-hydroxybenzotriazole), 1.20 equiv. HBTU (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphat), 2.4 equiv. DIPEA (*N*,*N*-diisopropylethylamine)). Being confirmed of successful coupling, the Fmoc protection group got removed using 20% DIPEA in DMF. After cleaving the peptide from the resin (77.5% TFA (thrifluoroacetate), 5% triisopropylsilane, 5% methylphenylsulfid, 5% phenol, 2.5% 1,2-ethandithiole, 5% water) the peptides were purified using HPLC chromatography. Purification was performed using a C4 10 x 250 mm column (Grace Vydac), exploiting individually optimised water-acetonitrile gradients (typically, 30-80%B over 20 min for the separating part of the gradient) at 35°C with a flow of 7 mL/min. Both eluents were supplemented with 5 mM HCl for ion-pairing. Purified peptides got identified by an LC/MS system equipped with an upstream 1100 Series LC-system from Agilent using a C4 reverse phase column (4,6 x 250 mm, Grace Vydac) at 35°C and a flow rate of 0.3 mL/min hyphenated to an ESI micro-TOFQ III mass spectrometer from Bruker.

EGFR$_{wt}$

NH_2–GPKIPSIATGMVGALLLLLVVALGIGLFMRRRHIV–COOH

LC/MS (ESI) (m/z): calcd for **EGFR$_{wt}$** [M+H$^+$]: 3683.6; [M+H$^+$]/2: 1841.8; [M+H$^+$]/3: 1227.9; [M+H$^+$]/4: 920.9; found: [M+H$^+$]/3: 1842.8935; [M+H$^+$]/2: 1228.9562; [M+H$^+$]/4: 921.9716.



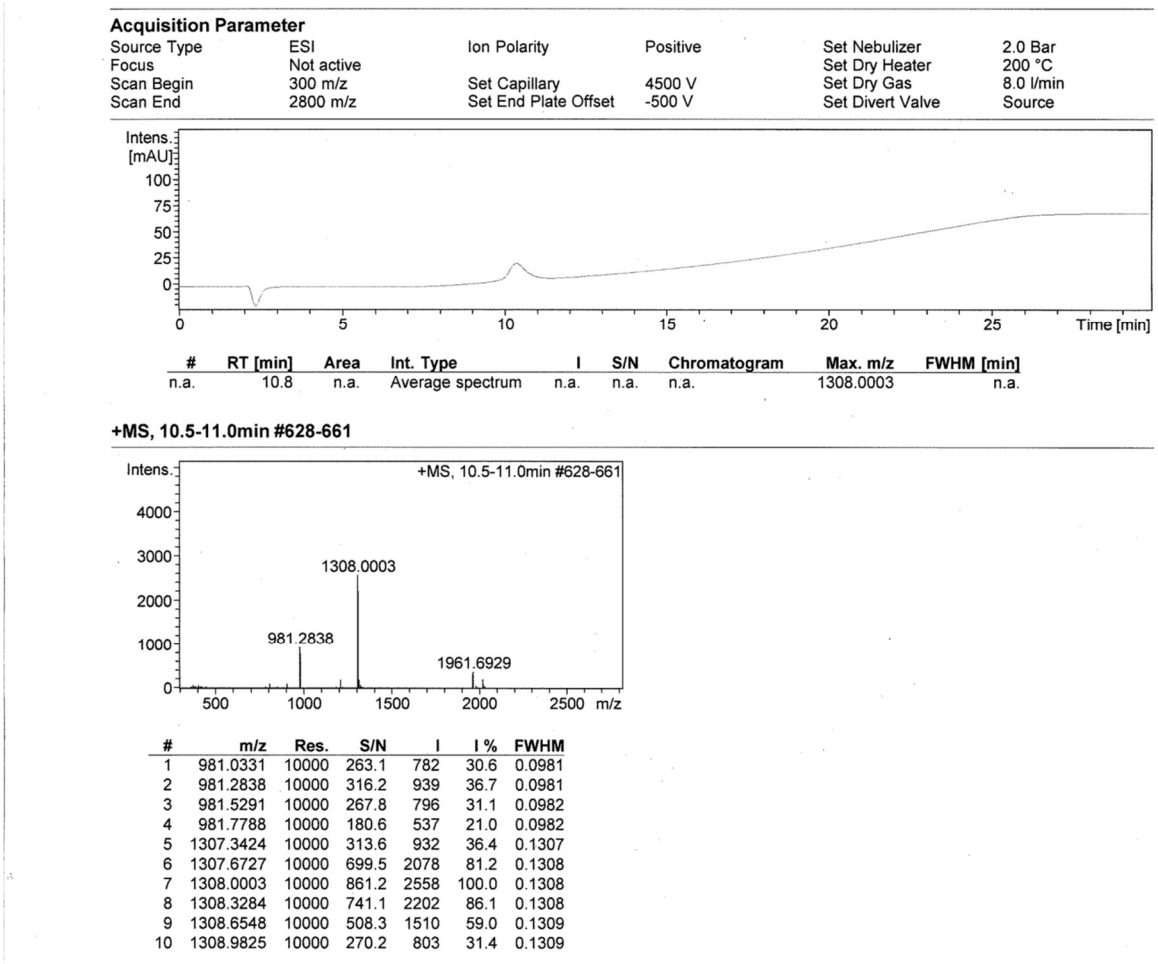
#	m/z	Res.	S/N	I	I %	FWHM
1	921.7214	10000	84622.7	33530	48.6	0.0922
2	921.9716	10000	94846.6	37581	54.5	0.0922
3	922.2211	10000	75468.0	29902	43.4	0.0922
4	922.4707	10000	48568.8	19244	27.9	0.0922
5	1228.2886	10000	74897.9	29677	43.0	0.1228
6	1228.6225	10000	155430.0	61585	89.3	0.1229
7	1228.9562	10000	174047.1	68962	100.0	0.1229
8	1229.2899	10000	139269.3	55182	80.0	0.1229
9	1229.6236	10000	86060.7	34100	49.4	0.1230
10	1842.8935	10000	48207.1	19101	27.7	0.1843

Figure S8: LC/MS (ESI) of **EGFR$_{wt}$**.

$EGFR_{FI}$

NH_2–**1**–KIPSIATGMVGALLLLLVVALGIGLFMRRRHIV–COOH

LC/MS (ESI) (m/z): calcd for **$EGFR_{FI}$** [M^+]: 3921; [M^+]/2: 1960.5; [M^+]/3: 1307; [M^+]/4: 980.3; found: [M^+]/2: 1961.69; [M^+]/3: 1308.00; [M^+]/4: 981.28.



Acquisition Parameter

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Scan End	2800 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

#	RT [min]	Area	Int. Type	I	S/N	Chromatogram	Max. m/z	FWHM [min]
n.a.	10.8	n.a.	Average spectrum	n.a.	n.a.	n.a.	1308.0003	n.a.

+MS, 10.5-11.0min #628-661

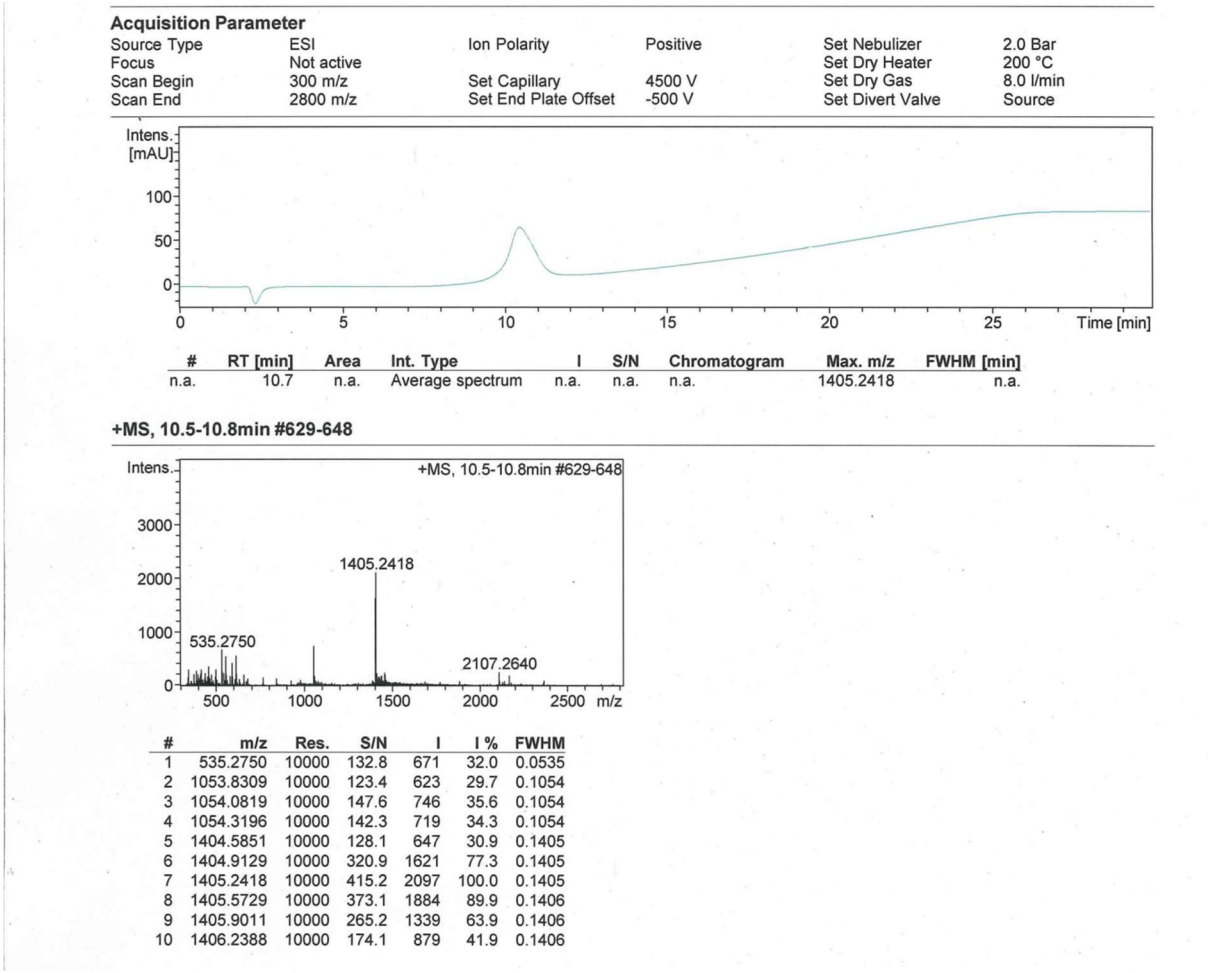
#	m/z	Res.	S/N	I	I %	FWHM
1	981.0331	10000	263.1	782	30.6	0.0981
2	981.2838	10000	316.2	939	36.7	0.0981
3	981.5291	10000	267.8	796	31.1	0.0982
4	981.7788	10000	180.6	537	21.0	0.0982
5	1307.3424	10000	313.6	932	36.4	0.1307
6	1307.6727	10000	699.5	2078	81.2	0.1308
7	1308.0003	10000	861.2	2558	100.0	0.1308
8	1308.3284	10000	741.1	2202	86.1	0.1308
9	1308.6548	10000	508.3	1510	59.0	0.1309
10	1308.9825	10000	270.2	803	31.4	0.1309

Figure S9: LC/MS (ESI) of **$EGFR_{FI}$**.

EGFR$_{FI}$1

NH_2–**1**–KIPS–**W**–ATGMVG–**W**–LLLLLV–**W**–ALGIGL–**Y**–MRRRHIV–COOH

LC/MS (ESI) (m/z): calcd for **EGFR$_{FI}$1** [M^+]: 4213; [M^+]/2: 2106.5; [M^+]/3: 1404.3; [M^+]/4: 1053.3; found: [M^+]/2: 2107.26; [M^+]/3: 1405.24; [M^+]/4: 1053.83.



#	m/z	Res.	S/N	I	I %	FWHM
1	535.2750	10000	132.8	671	32.0	0.0535
2	1053.8309	10000	123.4	623	29.7	0.1054
3	1054.0819	10000	147.6	746	35.6	0.1054
4	1054.3196	10000	142.3	719	34.3	0.1054
5	1404.5851	10000	128.1	647	30.9	0.1405
6	1404.9129	10000	320.9	1621	77.3	0.1405
7	1405.2418	10000	415.2	2097	100.0	0.1405
8	1405.5729	10000	373.1	1884	89.9	0.1406
9	1405.9011	10000	265.2	1339	63.9	0.1406
10	1406.2388	10000	174.1	879	41.9	0.1406

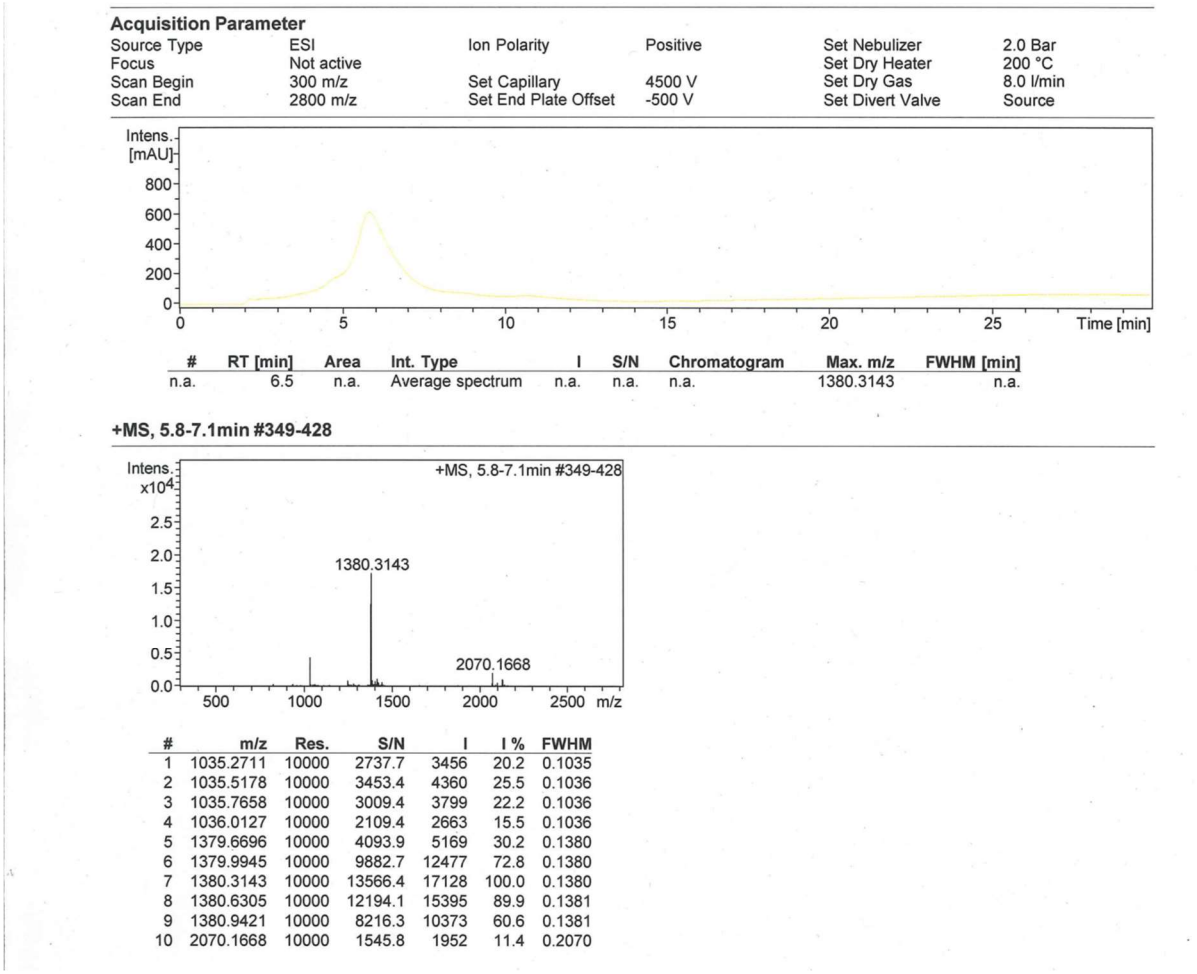
Figure S10: LC/MS (ESI) of **EGFR$_{FI}$1**.

.

EGFR$_{Fl}$2

NH_2–**1**–K–**W**–PSIATG–**W**–VGALLL–**W**–LVVALGIGL–**Y**–MRRRHIV–COOH

LC/MS (ESI) (m/z): calcd for **EGFR$_{Fl}$2** [M^+]: 4138.6; [M^+]/2: 2069.3; [M^+]/3: 1379.5; [M^+]/4: 1034.7; found: [M^+]/2: 2070.17; [M^+]/3: 1380.31.



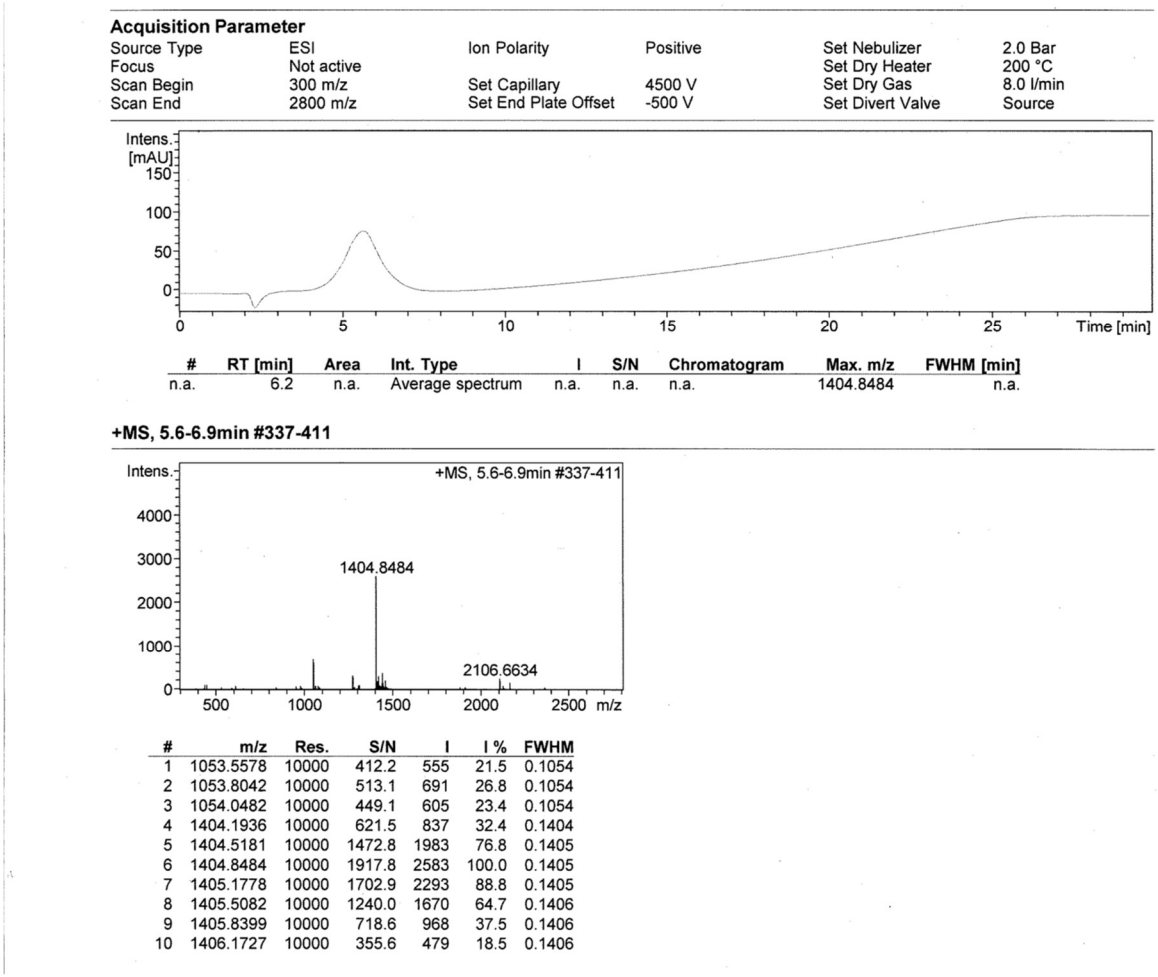
#	m/z	Res.	S/N	I	I %	FWHM
1	1035.2711	10000	2737.7	3456	20.2	0.1035
2	1035.5178	10000	3453.4	4360	25.5	0.1036
3	1035.7658	10000	3009.4	3799	22.2	0.1036
4	1036.0127	10000	2109.4	2663	15.5	0.1036
5	1379.6696	10000	4093.9	5169	30.2	0.1380
6	1379.9945	10000	9882.7	12477	72.8	0.1380
7	1380.3143	10000	13566.4	17128	100.0	0.1380
8	1380.6305	10000	12194.1	15395	89.9	0.1381
9	1380.9421	10000	8216.3	10373	60.6	0.1381
10	2070.1668	10000	1545.8	1952	11.4	0.2070

Figure S11: LC/MS (ESI) of **EGFR$_{Fl}$2**.

EGFR$_{Fl}$3

NH_2–**1**–K–**W**–PSIATG–**W**–VGALLL–**W**–LVVALG–**W**–GL–**Y**–MRRRHIV–COOH

LC/MS (ESI) (m/z): calcd for **EGFR$_{Fl}$3** [M^+]: 4211.6; [M^+]/2: 2105.8; [M^+]/3: 1403.9; [M^+]/4: 1052.9; found: [M^+]/2: 2106.66; [M^+]/3: 1404.85.



Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	2.0 Bar
Focus	Not active			Set Dry Heater	200 °C
Scan Begin	300 m/z	Set Capillary	4500 V	Set Dry Gas	8.0 l/min
Scan End	2800 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

#	RT [min]	Area	Int. Type	I	S/N	Chromatogram	Max. m/z	FWHM [min]
n.a.	6.2	n.a.	Average spectrum	n.a.	n.a.	n.a.	1404.8484	n.a.

+MS, 5.6-6.9min #337-411

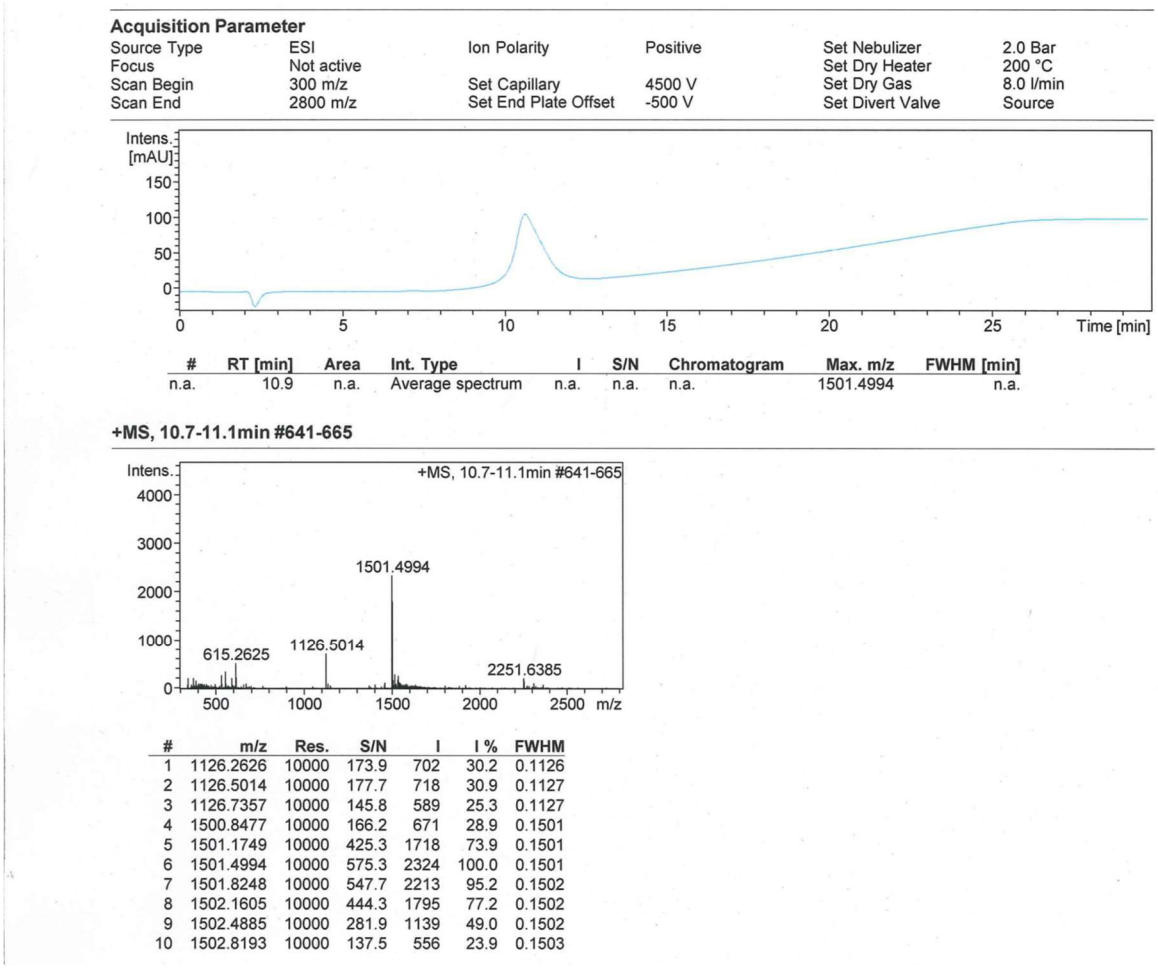
#	m/z	Res.	S/N	I	I %	FWHM
1	1053.5578	10000	412.2	555	21.5	0.1054
2	1053.8042	10000	513.1	691	26.8	0.1054
3	1054.0482	10000	449.1	605	23.4	0.1054
4	1404.1936	10000	621.5	837	32.4	0.1404
5	1404.5181	10000	1472.8	1983	76.8	0.1405
6	1404.8484	10000	1917.8	2583	100.0	0.1405
7	1405.1778	10000	1702.9	2293	88.8	0.1405
8	1405.5082	10000	1240.0	1670	64.7	0.1406
9	1405.8399	10000	718.6	968	37.5	0.1406
10	1406.1727	10000	355.6	479	18.5	0.1406

Figure S12: LC/MS (ESI) of **EGFR$_{Fl}$3**.

EGFR$_{Fl}$4

NH_2–**1**–K–**W**–PSI–**W**–TGM–**W**–GAL–**W**–LLL–**W**–VAL–**W**–IGL–**Y**–MRRRHIV–COOH

LC/MS (ESI) (m/z): calcd for **EGFR$_{Fl}$4** [M^+]: 4502; [M^+]/2: 2251; [M^+]/3: 1500; [M^+]/4: 1125; found: [M^+]/2: 2251.64; [M^+]/3: 1501.50; [M^+]/4: 1126.50.



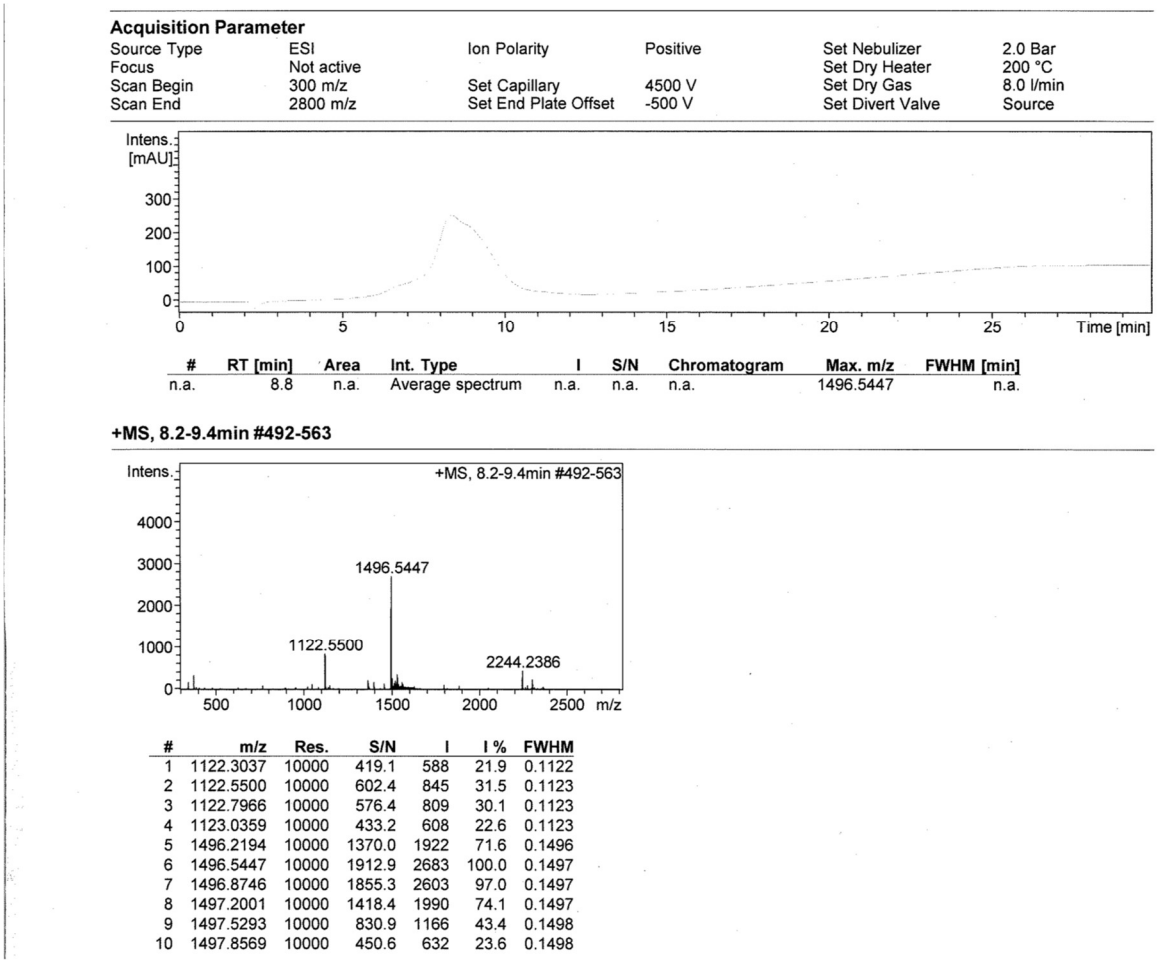
#	m/z	Res.	S/N	I	I %	FWHM
1	1126.2626	10000	173.9	702	30.2	0.1126
2	1126.5014	10000	177.7	718	30.9	0.1127
3	1126.7357	10000	145.8	589	25.3	0.1127
4	1500.8477	10000	166.2	671	28.9	0.1501
5	1501.1749	10000	425.3	1718	73.9	0.1501
6	1501.4994	10000	575.3	2324	100.0	0.1501
7	1501.8248	10000	547.7	2213	95.2	0.1502
8	1502.1605	10000	444.3	1795	77.2	0.1502
9	1502.4885	10000	281.9	1139	49.0	0.1502
10	1502.8193	10000	137.5	556	23.9	0.1503

Figure S13: LC/MS (ESI) of **EGFR$_{Fl}$4**.

EGFR$_{Fl}$5

NH_2–**1**–K–**W**–PS–**W**–ATG–**W**–VG–**W**–LLL–**W**–LV–**W**–ALG–**W**–GL–**Y**–MRRRHIV–COOH

LC/MS (ESI) (m/z): calcd for **EGFR$_{Fl}$5** [M^+]: 4486; [M^+]/2: 2243; [M^+]/3: 1495; [M^+]/4: 1121; found: [M^+]/2: 2244.24; [M^+]/3: 1496.54; [M^+]/4: 1122.55.



Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	2.0 Bar
Focus	Not active			Set Dry Heater	200 °C
Scan Begin	300 m/z	Set Capillary	4500 V	Set Dry Gas	8.0 l/min
Scan End	2800 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

#	RT [min]	Area	Int. Type	I	S/N	Chromatogram	Max. m/z	FWHM [min]
n.a.	8.8	n.a.	Average spectrum	n.a.	n.a.	n.a.	1496.5447	n.a.

+MS, 8.2-9.4min #492-563

#	m/z	Res.	S/N	I	I %	FWHM
1	1122.3037	10000	419.1	588	21.9	0.1122
2	1122.5500	10000	602.4	845	31.5	0.1123
3	1122.7966	10000	576.4	809	30.1	0.1123
4	1123.0359	10000	433.2	608	22.6	0.1123
5	1496.2194	10000	1370.0	1922	71.6	0.1496
6	1496.5447	10000	1912.9	2683	100.0	0.1497
7	1496.8746	10000	1855.3	2603	97.0	0.1497
8	1497.2001	10000	1418.4	1990	74.1	0.1497
9	1497.5293	10000	830.9	1166	43.4	0.1498
10	1497.8569	10000	450.6	632	23.6	0.1498

Figure S14: LC/MS (ESI) of **EGFR$_{Fl}$5**.

4. Spectroscopic data

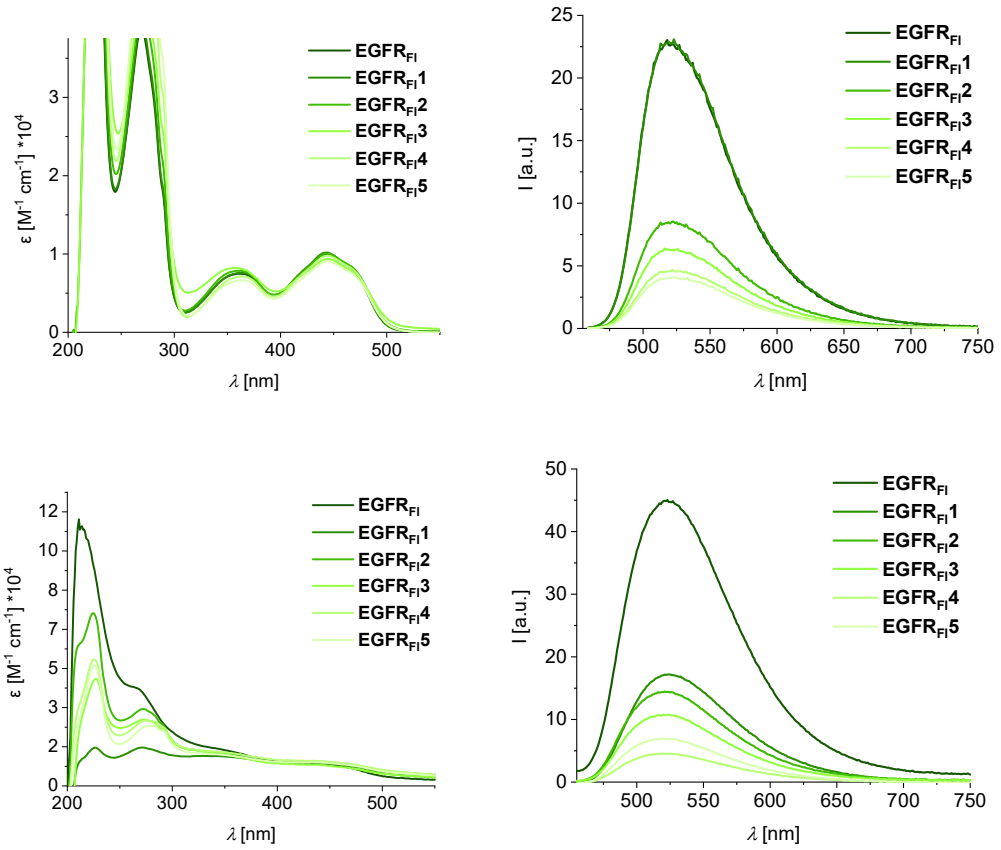


Figure S15: *Absorption and fluorescence of* ***EGFR$_{Fl}$-EGFR$_{Fl}$5*** *in MeCN/H2O 1:1 ($\lambda_{exc.}$ = 440 nm; 0.04 mM) and POPC vesicle system ($\lambda_{exc.}$ = 440 nm; 0.012 mM flavin).*

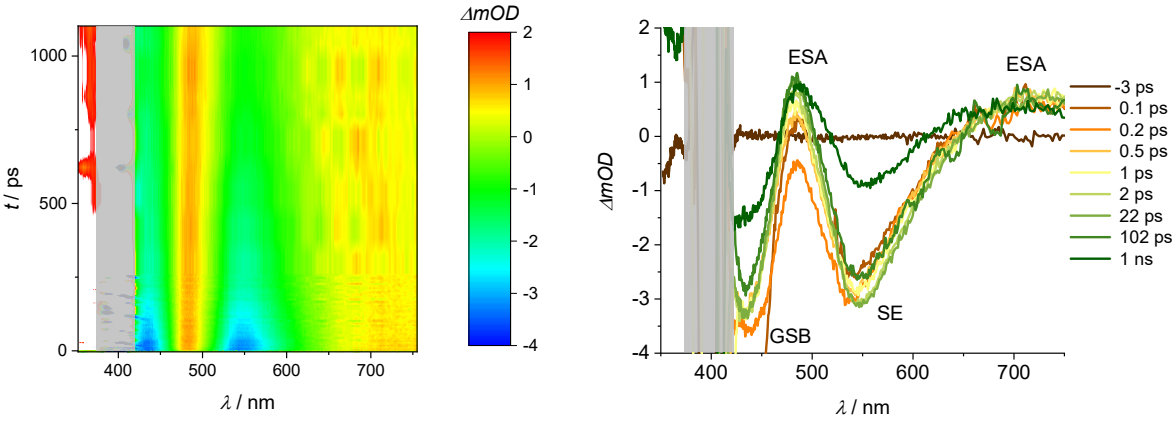


Figure S16: Contour plot of **EGFR$_{Fl}$** (left) and transient absorption spectra (right). λ_{ex} = 400 nm, E = 2 µJ, $OD_{400\,nm}$ = 0.17. GSB = ground state bleach, ESA = excited state absorption, SE = stimulated emission.

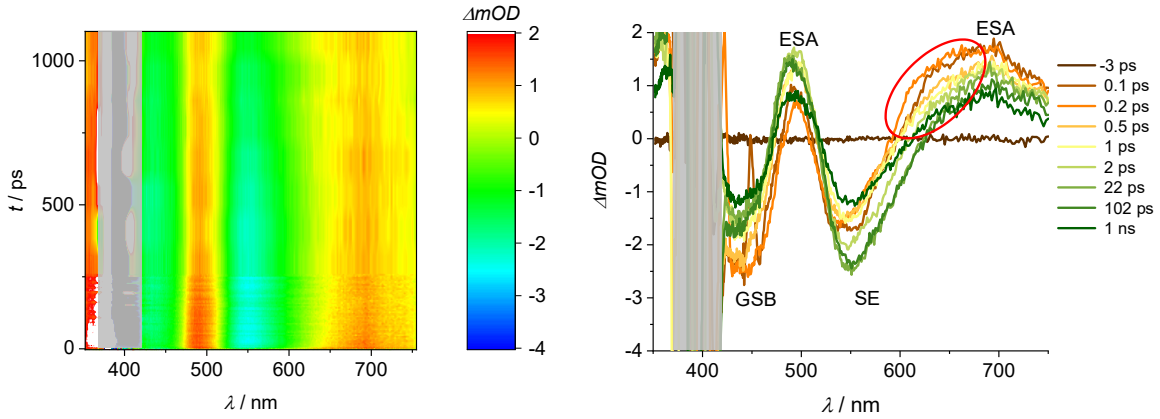


Figure S17: Contour plot of **EGFR$_{Fl}$2** (left) and transient absorption spectra (right). λ_{ex} = 400 nm, E = 2 µJ, $OD_{400\ nm}$ = 0.34. GSB = ground state bleach, ESA = excited state absorption, SE = stimulated emission. The red circle indicates the spectral region of interest that is similar in **EGFR$_{Fl}$2-EGFR$_{Fl}$5**, but does not exist in the reference system **EGFR$_{Fl}$**.

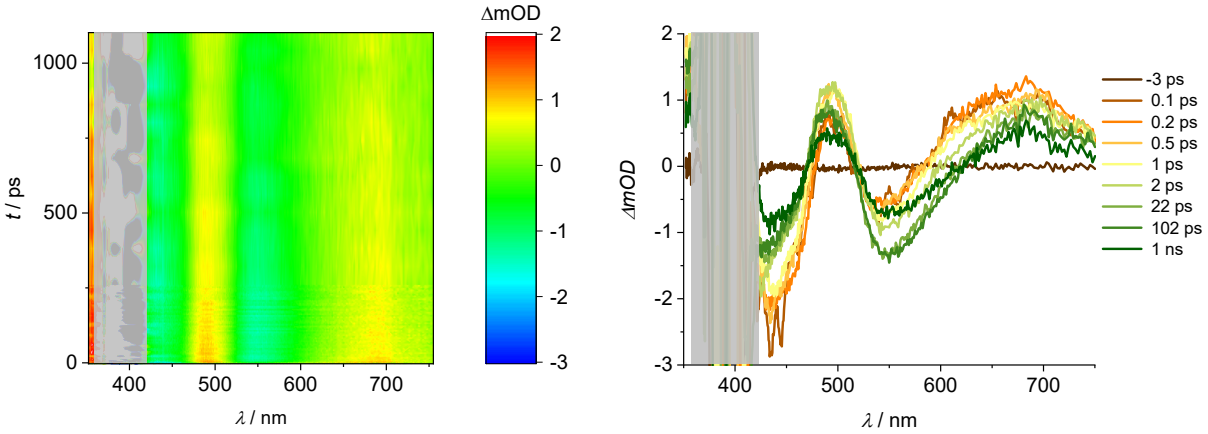


Figure S18: Contour plot of **EGFR$_{Fl}$3** (left) and transient absorption spectra (right). λ_{ex} = 400 nm, E = 2 µJ, $OD_{400\ nm}$ = 0.23.

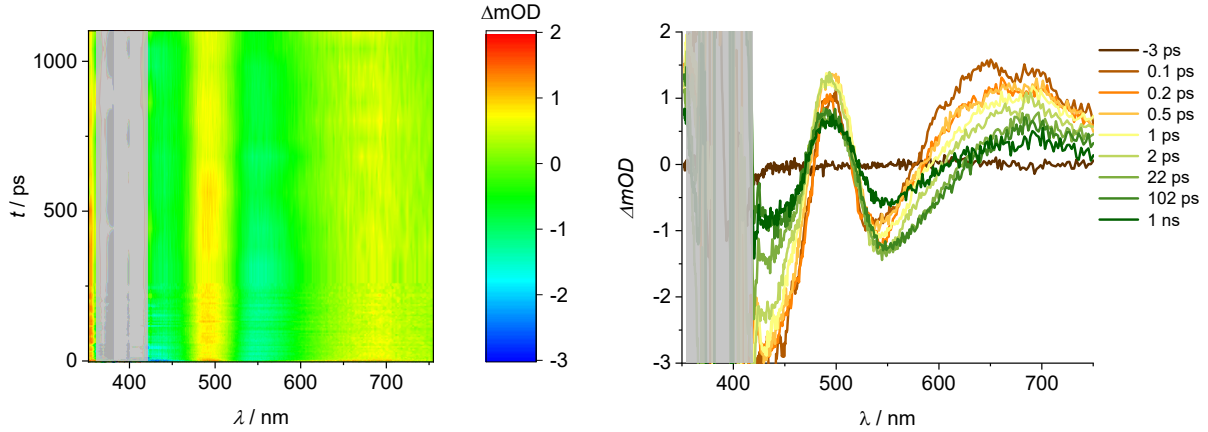


Figure S19: Contour plot of **EGFR$_{Fl}$4** (left) and transient absorption spectra (right). λ_{ex} = 400 nm, E = 2 µJ, $OD_{400\ nm}$ = 0.29.

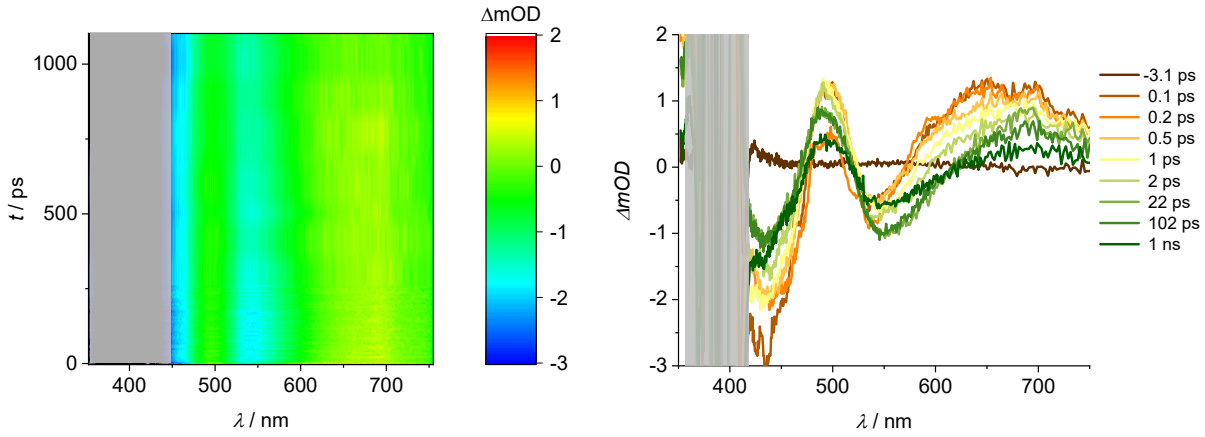


Figure S20: Contour plot of **EGFR$_{Fl}$5** (left) and transient absorption spectra (right). λ_{ex} = 400 nm, E = 2 µJ, $OD_{400\ nm}$ = 0.21.

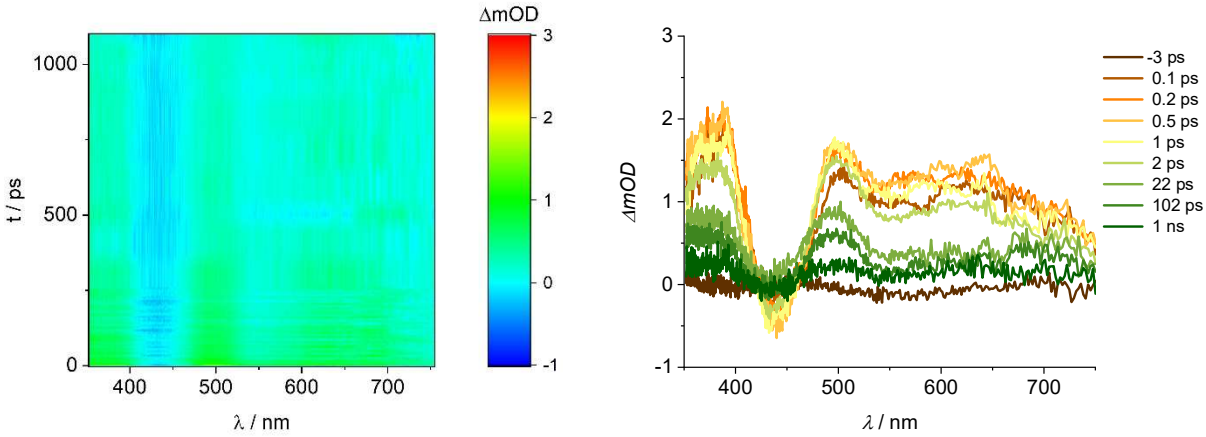


Figure S21: Contour plot of **EGFR$_{Fl}$5** (left) and transient absorption spectra (right). λ_{ex} = 267 nm, E = 0.5 µJ, $OD_{267\ nm}$ = 1.93.

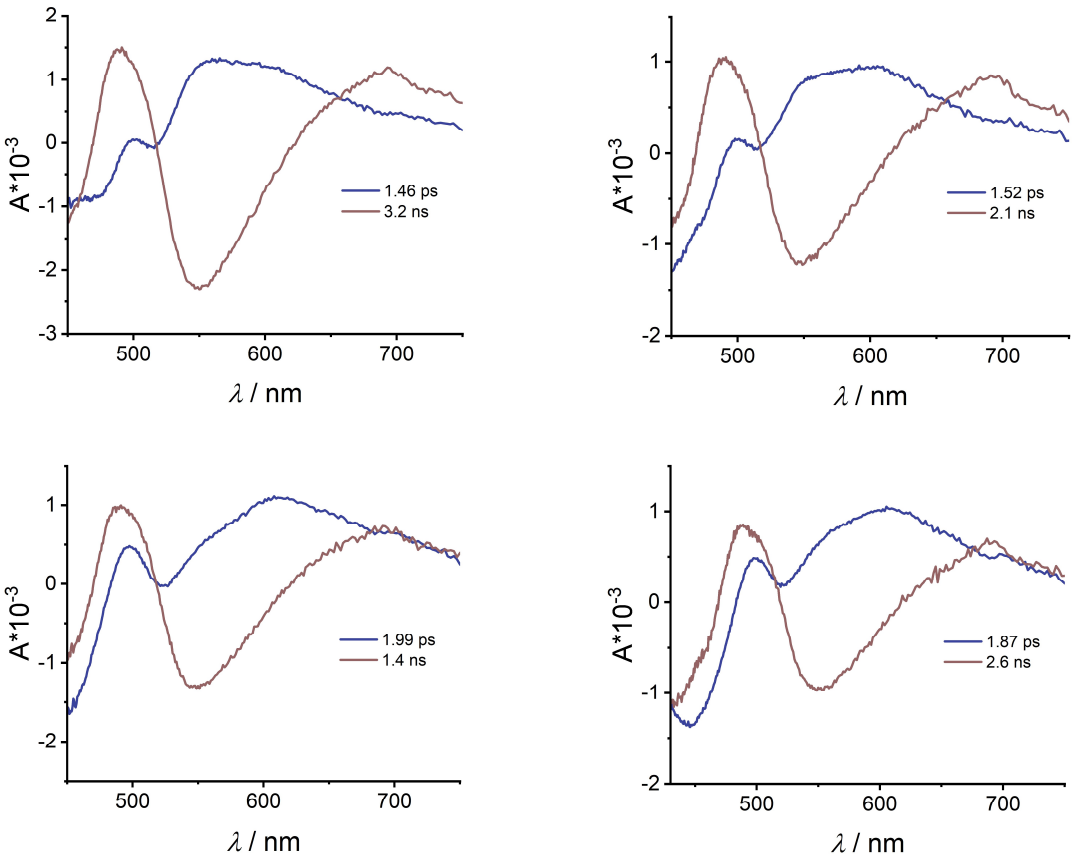


Figure S21: DADS (blue, brown) of $EGFR_{FL}2$-5.

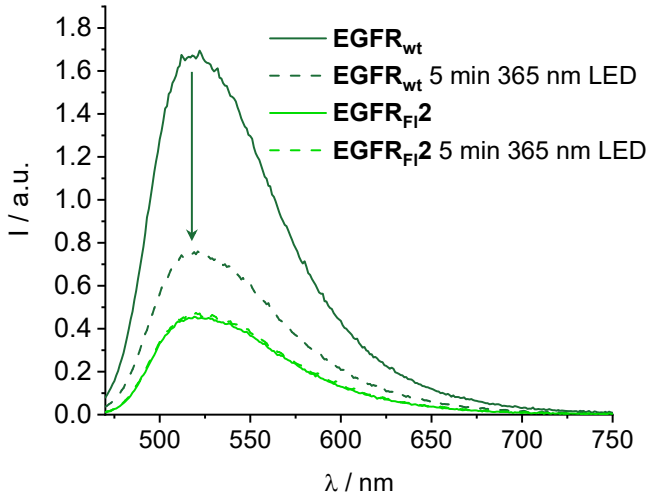


Figure S22. Fluorescence of **$EGFR_{wt}$** and **$EGFR_{Fl}2$** (0.04 mM) in MeCN:H_2O (1:1) mixtures, λ_{exc}=440 nm, before and after irradiation by the 365 nm LED.

5. Literature

[1] G. B. Fields, R. L. Noble, *Int. J. Pept. Protein Res.* 1990, *35*, 161-214.

[2] P. Wadhwani, E. Strandberg, J. van den Berg, C. Mink, J. Burck, R. A. Ciriello, A. S. Ulrich, *BBA* 2014, *1838*, 940-949.

[3] J. Bürck, S. Roth, P. Wadhwani, S. Afonin, N. Kanithasen, E. Strandberg, A. S. Ulrich, *Biophys. J.* 2008, *95*, 3872-3881.

[4] R. Kumar, R. K. Arigela, S. Samala, B. Kundu, *Chem. Eur. J.* 2015, *21*, 18828-18833.