Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Arginine-Selective Bioconjugation with 4-Azidophenyl glyoxal: Application to the Single and Dual Functionalisation of Native Antibodies

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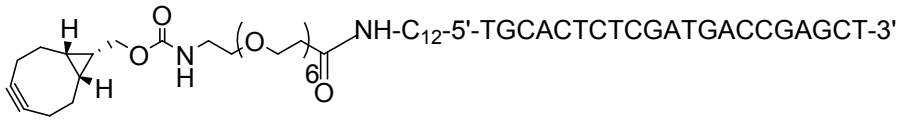
Compounds synthesis

Materials: All reagents were obtained from commercial sources and used without prior purifications. Dry solvents were obtained from Sigma-Aldrich. 4-Azidophenyl glyoxal hydrate (APG) was purchased from Apollo Scientific. Cy5-NHS was purchased from Lumiprobe. TAMRA-BCN was synthesized as previously described.[1]

Preparation of BCN-ON1



MW = 7157.1 g/mol

Oligonucleotide-BCN **ON1** was prepared according to previously described protocol.[1] The purity of the **ON1** was evaluated by HPLC using a reaction with 1.3 eq. of TAMRA-N_3 as shown on Figure 1S. The detection was done at 260 nm.

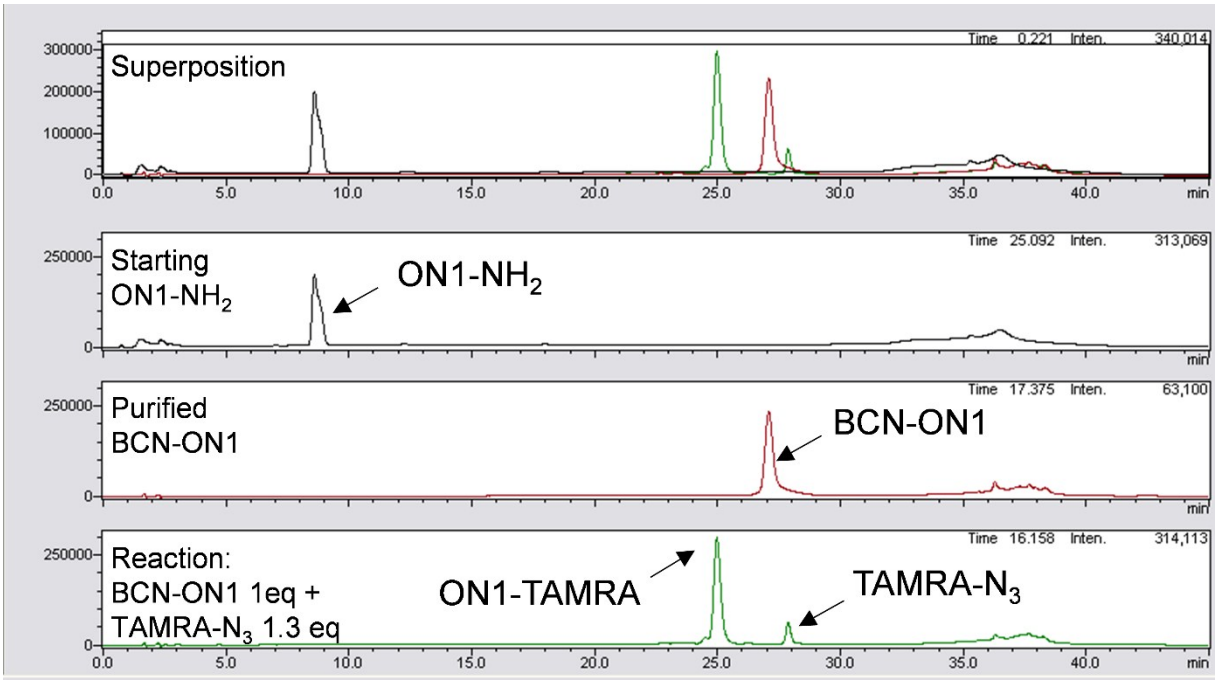


Figure 1S. Characterization of **BCN-ON1** purity by HPLC (detection at 260 nm) using a reaction with 1.3 eq. of TAMRA-N_3.

Structure of ON2

NH_2-C_{12}-5'-AGC TCG GTC ATC GAG AGT GCA-3'

MW = 6734.6 g/mol

Chemical biology

General experimental procedures: Protein concentration of antibody stock solution (PBS 1/20X, pH 7.5) was determined by UV absorbance using a NanoDrop spectrophotometer

(Thermo Fisher Scientific, Illkirch, France). The concentration of antibody conjugates was measured using BCA Protein Assay Kit (Ref. 23225, Thermo Fisher Scientific).

Preparation of antibody conjugates

Preparation of T-N_3(R) conjugates for evaluation of APG efficiency. APG (2, 5, 10 or 20 eq. in 6.86 µL of DMSO) was added to a solution of trastuzumab (1 eq., 5 mg/mL, 100 µL) in PBS (1x, pH 7.5) at 4 °C and the reaction mixture was incubated at 25 °C for 16 h. The excess of reagents was then removed by gel filtration chromatography using Bio-spin P-30 Columns (Bio-Rad, Hercules, U.S.A.) pre-equilibrated with PBS (1x, pH 7.5) to give a solution of trastuzumab-azide conjugates **T-N_3(R)**, which were subjected to native-HRMS for a measurement of the average degree of conjugation (DoC). The DoC values (triplicates) were plotted vs amount of added APG reagent as present on Figure 2C of the main article.

Preparation of antibody-oligonucleotide conjugates. A solution of **T-N_3(R)** conjugates (from step above, 2 mg/mL, 50 µL in PBS (1x, pH 7.5)) with DoC (0.7, 1.5 or 2.9) was treated with **BCN-ON1** (1.3 eq. per azide groups, 249 µM in H_2O) at 25 °C for 18 h. The excess of reagents was then removed by gel filtration chromatography using Bio-spin P-30 Columns pre-equilibrated with PBS 1/20x (pH 7.5) to give a solution of trastuzumab-oligo conjugates **T-ON1**, which were characterized by SDS PAGE (at 0.1 mg/mL Ab). The hybridization to **T-ON1-ON2** was evaluated by SDS PAGE analysis by preparing SDS PAGE sample of **T-ON1** (1 eq., 0.1 mg/mL, 24 µL) containing 6 eq. (98 pmol) of complementary oligonucleotide **ON2.**

Dual orthogonal antibody functionalisation. APG (8 eq., 2.82 µL, 10 mM in DMSO) and Cy5-NHS (4 eq., 3.44 µL, 4.1 mM in DMSO) were added to a solution of trastuzumab (1 eq., 5.15 mg/mL, 100 µL) in PBS (1x, pH 7.5) and the reaction mixture was incubated at 25 °C for 18 h. The excess of reagents was then removed by gel filtration chromatography using Bio-spin P-30 Columns pre-equilibrated with PBS (1x, pH 7.5) to give **T-N_3(R)Cy5(K)** conjugate, which were reacted with TAMRA-BCN (5 eq., 15 mM in DMSO) for 18 h. After gel filtration chromatography using Bio-spin P-30 columns the **T-TAMRA(R)-Cy5(K)** conjugate was analysed by UV-Vis spectroscopy and in-gel fluorescence imaging (see Figure 7 B,C). In parallel, **T-TAMRA(R)** and **T-Cy5(K)** conjugates were prepared using the similar protocol and the same amount of reagents (APG 8 eq., Cy5-NHS 4 eq., TAMRA-BCN 5 eq. per 1 eq. of mAb).

Selectivity of APG towards arginine residues.

APG (5 eq. (1.72 µL), 8 eq. (2.75 µL) or 11 eq. (3.78 µL), 10 mM in DMSO) was added to a solution of trastuzumab (1 eq., 5 mg/mL, 100 µL) in PBS (1x, pH 7.5) at 4 °C. In a parallel test, a solution of L-arginine or L-lysine (2900 eq., 10 µL, 1 M) in PBS buffer (1x, adjusted to pH 7.5 by 1M HCl solution) was added to a solution of trastuzumab (1 eq., 5 mg/mL, 100 µL) and then APG (5 eq., 1.72 µL, 10 mM in DMSO) was added at 4 °C. The samples were incubated at 25 °C for 16 h. The excess of reagents was then removed by gel filtration chromatography using Bio-spin P-30 Columns pre-equilibrated with PBS 1/20x (pH 7.5) to give a solution of trastuzumab-azide conjugates. The resulting conjugates were subjected to SPAAC with TAMRA-BCN (5 eq., 1.14 µL, 15 mM in DMSO) at 25 °C for 18 h. As a negative control, a

solution of trastuzumab (1 eq, 5 mg/mL, 100 µL) was treated with TAMRA-BCN (5 eq., 1.14 µL, 15 mM in DMSO) at 25 °C for 18 h. The excess of the reagent was then removed by gel filtration chromatography using Bio-spin P-30 Columns pre-equilibrated with PBS 1/20x (pH 7.5) to yield (70-90%) **T-TAMRA(R)** conjugates, which were analysed by SDS PAGE (at 0.5 mg/mL Ab) and native-HRMS (samples based on 8 eq. and 11eq. of APG).

Kinetic study of antibody modification with APG

One after another DMSO (10 µL) and APG (11 eq., 25.14 µL, 15 mM in DMSO) were added to a solution of trastuzumab (1 eq, 5 mg/mL, 1000 µL) in PBS 1x (pH 7.5) at 25 °C and the reaction mixture was incubated at 25 °C. At certain time points (1 h, 2 h, 4 h, 6 h, 9 h and 32 h) aliquots (100 µL) were taken and purified by gel filtration chromatography using Bio-spin P-30 Columns pre-equilibrated with PBS 1/20x (pH 7.5). The resulting conjugates were subjected to SPAAC with TAMRA-BCN (5 eq., 1.14 µL, 15 mM in DMSO) at 25 °C for 18 h. The excess of the reagent was then removed by gel filtration chromatography using Bio-spin P-30 Columns pre-equilibrated with PBS 1/20x (pH 7.5) to yield (70-95%) **T-TAMRA(R)** conjugates. Concentration of the conjugates was adjusted to 0.5 mg/mL and absorption at 558 nm was measured in a quartz cuvette (chamber volume 160 µL, ref. Z600318, Sigma-Aldrich) using UV-Vis spectrophotometer (Varian Cary 100 Bio). The resulting absorption values were plotted vs time as present on Figure 3B of the main article.

Stability of T-TAMRA(R) in human plasma

Four aliquots of **T-TAMRA(R)** conjugates (65 µL, 5 mg/mL) were buffer exchanged using Bio-spin P-30 Columns pre-equilibrated with different buffers: 1) PBS (1x, pH 6.5), 2) PBS (1x, pH 7.5), 3) PBS (1x, pH 8.5) and 4) PBS (1x, pH 7.5). To the probe 4 solution of NH_2OH (0.5 M in PBS 1/2x, adjusted to pH 7.5) was added to get final PBS buffer (1x, pH 7.5) containing 10 mM of hydroxylamine. For all four probes the DoC (UV) = 3.0 ± 0.1. The probes were incubated at 25 °C for 24 h and then buffer exchanged using Bio-spin P-30 Columns pre-equilibrated with PBS (1x, pH 7.5). For all four probes the DoC (UV) became 2.8 ± 0.1. The probes (1 mg/mL, 50 µL) were mixed with human plasma (50 µL) and incubated at 37 °C. Every 24 h aliquots (2 µL) were taken, diluted with water (98 µL) and stored at -20 ˚C. The resulting samples (24 µL) were then subjected to SDS PAGE analysis (Figure 2S). Human plasma was supplied by Etablissement Français du Sang (EFS Strasbourg).

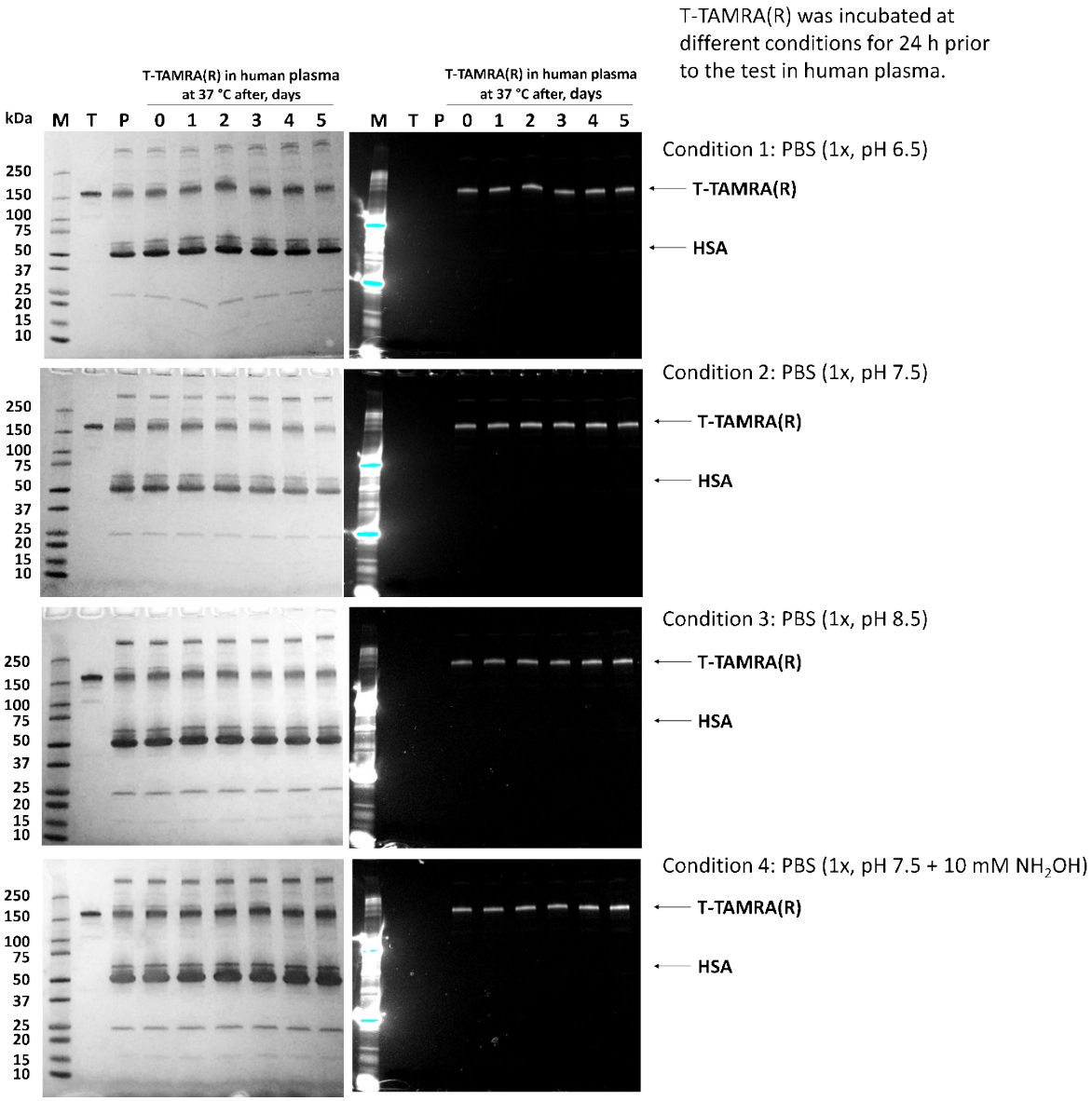


Figure 2S. Evaluation of **T-TAMRA(R)** stability in human plasma at 37 °C using SDS-PAGE analysis.

Characterization of conjugates

SDS PAGE analysis

Non-reducing glycine-SDS-PAGE was performed on 4–15% Mini-PROTEAN® TGX™ Gel (Ref. 4561084, Bio-Rad) following standard lab procedures. To samples containing antibody conjugates (0.1 mg/mL or 0.5 mg/mL, 24 µL in H_2O) was added 8 µL of 4x non-reducing Laemmli SDS sample buffer ((Ref. J63615, Alfa Aesar) and heated at 95 °C for 3 minutes. The gel was run at constant voltage (200 V) for 40 min using TRIS 0.25 M - Glycine 1.92 M - SDS 1% as a running buffer. Fluorescence was visualized on ImageQuant LAS 4000 series (GE Healthcare Life Sciences) prior to staining with Coomassie Blue.

Samples preparation for HRMS analysis

Prior to high resolution native mass spectrometry (native-HRMS) experiments, deglycosylated antibody conjugates (AC) were desalted against 150 mM ammonium acetate solution buffered at pH 7.4 using six cycles of concentration/dilution on micro-concentrators (Vivaspin, 30 kD cutoff, Sartorius, Gottingen, Germany). AC deglycosylation was achieved by incubating (37 °C – 2 h) 0.4 units of Remove-iT® Endo S (New England Biolabs, Ipswich, U.S.A.) per microgram of AC prior to the buffer exchange desalting step. Protein concentration was determined by UV absorbance using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Illkirch, France).

HRMS analysis

Native-HRMS was performed on an Exactive Plus EMR (Thermo Fisher Scientific, Bremen, Germany) coupled to an automated chip-based nanoelectrospray device (Triversa Nanomate, Advion, Ithaca, USA). Electrospray ionization was conducted at a capillary voltage of 1.86 kV and nitrogen nanoflow of 0.15 psi.

Native-HRMS experiments were performed using classical interface tuning parameters of the mass spectrometer with a nominal resolution of either 17,500 or 35,000 and in the positive ion mode. The in-source collision-induced dissociation and the higher-energy collisional dissociation cells were set to 200 eV and 50 eV respectively. The trapping gas pressure was set to 3 a.u. (which corresponds to an Ultra High Vaccum of 4.10^{-10} mbar approximatively). In order to improve the transmission of the high mass species the voltages on the injection, inter and bent flatapole were fixed to 8, 7 and 6 V respectively.

External calibration was performed using singly-charged ions produced by a 2 mg/mL solution of cesium iodide in 2-propanol/water (50/50 v/v) and samples were infused at 5 µM in NH_4OAc 150 mM pH 7.5. MS data interpretations and deconvolutions were performed using Protein Deconvolution 4.0 available on BiopharmaFinder SP1 (Thermo Fisher, Bremen, Germany). The parameters of software were optimized for each spectrum.

DoC calculation from MS analysis

The average Degree of Conjugation (DoC) values from native MS were calculated by using Eq. 1. These results were derived from the relative peak intensities in deconvoluted mass spectra.

$$DoC = \frac{\sum_{k=0}^{k=n} k * I(DoC_k)}{\sum_{k=0}^{k=n} I(DoC_k)} \quad \textbf{Eq.1}$$

where $I(DoC_k)$ is relative peak intensity of conjugates with k add-on molecules per antibody.

DoC calculation from UV-Vis spectroscopy

The average Degree of Conjugation (DoC) values from UV-Vis spectra were calculated by using Eq. 2-3 and next correction factors (CF) of the fluorophores: CF_{280}(TAMRA) = 0.2,

CF_{280}(Cy5) = 0.05, CF_{555}(Cy5) = 0.1 and extinction coefficients: ε_{555}(TAMRA)= 65 000 L/(mole·cm), ε_{650}(Cy5) = 250 000 L/(mole·cm), ε_{280}(IgG) = 203 000 L/(mole·cm).

Eq. 2 $$DoC(TAMRA) = \frac{\left(A_{555} - CF_{555}^{Cy5} \cdot A_{650}\right)}{A_{280} - \left(A_{555} - CF_{555}^{Cy5} \cdot A_{650}\right) \cdot CF_{280}^{TAMRA} - A_{650} \cdot CF_{280}^{Cy5}} \times \frac{\varepsilon_{280}^{IgG}}{\varepsilon_{555}^{TAMRA}}$$

Eq. 3 $$DoC(Cy5) = \frac{A_{650}}{A_{280} - \left(A_{555} - CF_{555}^{Cy5} \cdot A_{650}\right) \cdot CF_{280}^{TAMRA} - A_{650} \cdot CF_{280}^{Cy5}} \times \frac{\varepsilon_{280}^{IgG}}{\varepsilon_{650}^{Cy5}}$$

where A_{280}, A_{555} and A_{650} are absorptions at 280, 555 and 650 nm, respectively.

Sample preparation for peptide mapping analysis

Fifteen micrograms of deglycosylated antibody conjugates was solubilized in 150 mM NH_4HCO_3, 0.1% RapiGest™ (Waters, Milford, USA) at pH 7.4. Disulfide reduction was performed by incubating the antibody conjugate solution with 5 mM DTT for 30 min at 60 °C. Alkylation was performed with 15 mM IAA for 30min in the dark. After these steps, the samples were split in two for enzymatic digestion using trypsin or pepsin.

Digestion was performed by adding trypsin (Promega, Madison, USA) to a 1:50 enzyme to substrate ratio. Samples were incubated overnight at 37 °C. The reaction was quenched by adding 1% of TFA. RapiGest™ was eliminated by centrifugation at 10 000 g for 5 min.

For pepsin digestion, pH was decreased to 2.0 prior to pepsin (Promega, Madison, USA) digestion. Digestion was performed by adding pepsin at a 1:50 enzyme to substrate ratio. Samples were incubated at 37 °C for 3h. The reaction was stopped by heating at 95 °C for 10 min. RapiGest™ was eliminated by a centrifugation at 10 000 g for 5 min.

Peptide mapping analysis

NanoLC-MS/MS analysis was performed using a nanoAcquity Ulta-Performance-LC (Waters, Milford, USA) coupled to the TripleTOF 5600 mass spectrometer (Sciex, Ontario, Canada). The sample were trapped on a nanoACQUITY UPLC precolumn (C18, 180 µm x 20 mm, 5 µm particle size), and the peptides were separated on a nanoACQUITY UPLC column (C18, 75 µm x 250 mm with 1.7 µm particle size, Waters, Milford, USA). Mobile phase A was 0.1% (v/v) formic acid in water and mobile phase B was 0.1% (v/v) formic acid in acetonitril. A gradient (3% B for 35 min, 3-85% B for 1 min, 85-3% B for 1 min, maintained 3% B for 13 min) was used at a flow rate of 300 nL/min. The TripleTOF 5600 was operated in the positive mode, with the following settings: ionspray voltage floating (ISVF) 2350 V, curtain gas (CUR) 25, interface heater temperature (IHT) 75, ion source gas 1 (GS1) 8, declustering potential (DP) 80 V. Information-dependent acquisition (IDA) mode was used with Top 10 MS/MS scans. The MS scan accumulation time was set to 250 ms on m/z range [300; 1250] and the MS/MS scans to 100 ms on the m/z range [100; 1800] in the high sensitivity mode. Switching criteria were set to ions with charge state of 2-4 and an abundance threshold of more than 250 counts, exclusion time was set at 8 s. IDA rolling collision energy script was used for automatically adapting the CE. Mass calibration of the analyser was achieved using peptides from digested BSA. The complete system was fully controlled by AnalystTF 1.7.1 (Sciex).

Conjugation sites identification

Raw data collected were processed and converted in .mgf format. The mgf files of the trypsin and pepsin digestion were merged using Mass Spectrometry Data Analysis 2.7.3 (MSDA).
The MS/MS data were interpreted using a local Mascot server with MASCOT 2.5.0 algorithm (Matrix Science, London, UK). Spectra were searched with a mass tolerance of 25 ppm for MS and 0.07 Da for MS/MS data, using none as enzyme. Carbamidomethylation of cysteine residues, oxidation of methionine residues and linker (+157 Da) added to the arginine of the mAb were specified as variable modifications. Protein identifications were validated with Mascot ion score above 25. Each conjugation sites was manually validate based on the presence of y-ion and b-ion series and the peak intensity observed on the MS/MS spectra, using Proline 1.5 software.[2]

Average DoC	HC 19	HC 50	HC 59	HC 87	HC 258	HC 304	LC 24	LC 108
0.7		x	x			x		
1.4		x	x	x	x	x	x	X
3.1		x	x	x	x	x	x	X
5.3	x	x	x	x	x	x	x	X

Table S1. Conjugated arginine observed by peptide mapping analysis for sample with different average DoC.

Antigen recognition properties

The antigen recognition properties of the **T-TAMRA(R)** and **T-N_3(R)** conjugates was determined using flow cytometry on two breast adenocarcinoma cell lines: (i) $HER2^+$ SKBR-3 cells; (ii) $HER2^-$ MDA-MB-231 cells. A single cell suspension was obtained and the staining was performed as described earlier.[1] Briefly, the adherent cells were trypsinized at 37 °C and subsequent steps were performed at 4 °C. Unspecific epitopes were blocked and, subsequently the cells were incubated with the following primary antibodies/ADCs (20 µg/mL): trastuzumab, trastuzumab emtansine (T-DM1, DoC 3.6), **T-TAMRA(R)** (DoC 3.5), **T-N_3(R)** (DoC 3.6) or IgG1 isotype control, i.e. rituximab. Secondary antibody staining was performed with DyLight649-conjugated goat anti-human IgG antibody (Novus Biologicals, Littleton, CO, USA). The samples were analysed on the Guava® easyCyte 12HT (Merck Millipore, Molsheim, France) and the data analysis was performed using FlowJo X.0.7 (Tree Star, Ashland, OR, USA).

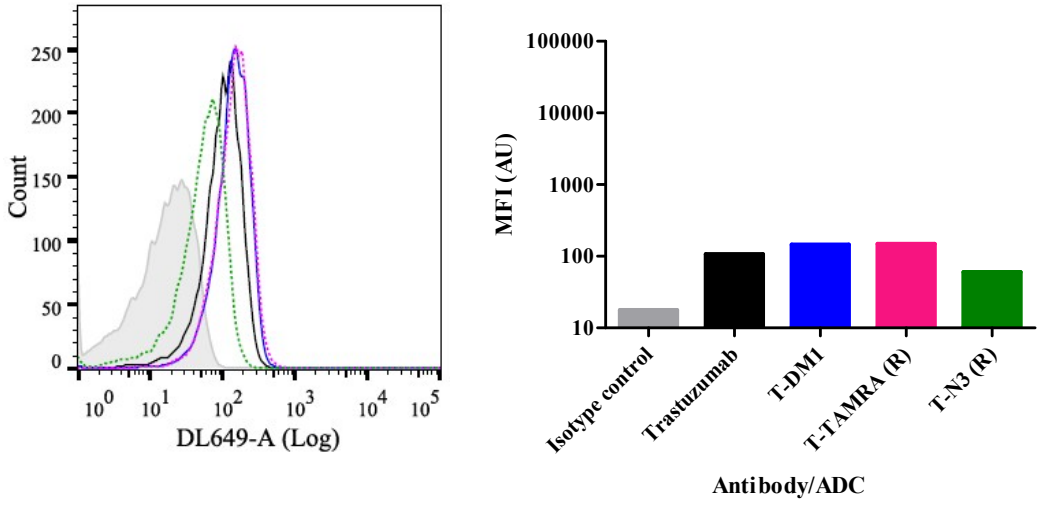
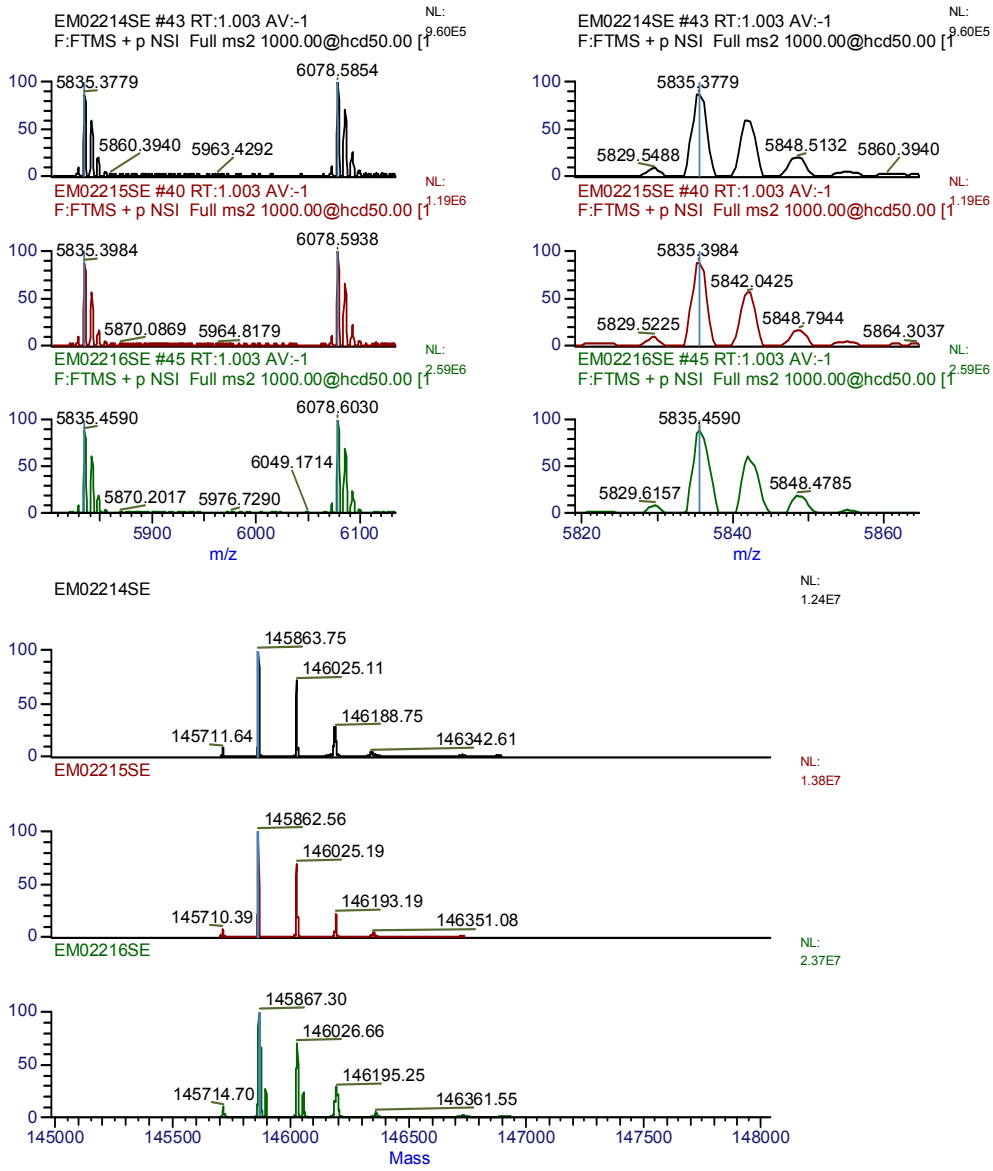


Figure 3S. Median fluorescence intensities (MFIs) of the native antibody trastuzumab (black), the reference trastuzumab emtansine **T-DM1** (blue), **T-TAMRA (R)** (pink) and **T-N_3(R)** in $HER2^-$ MDA-MB-231 cells. Rituximab was used as isotype control (grey). The scale of the bar-plot was adapted to that of Figure 5B of the main article.

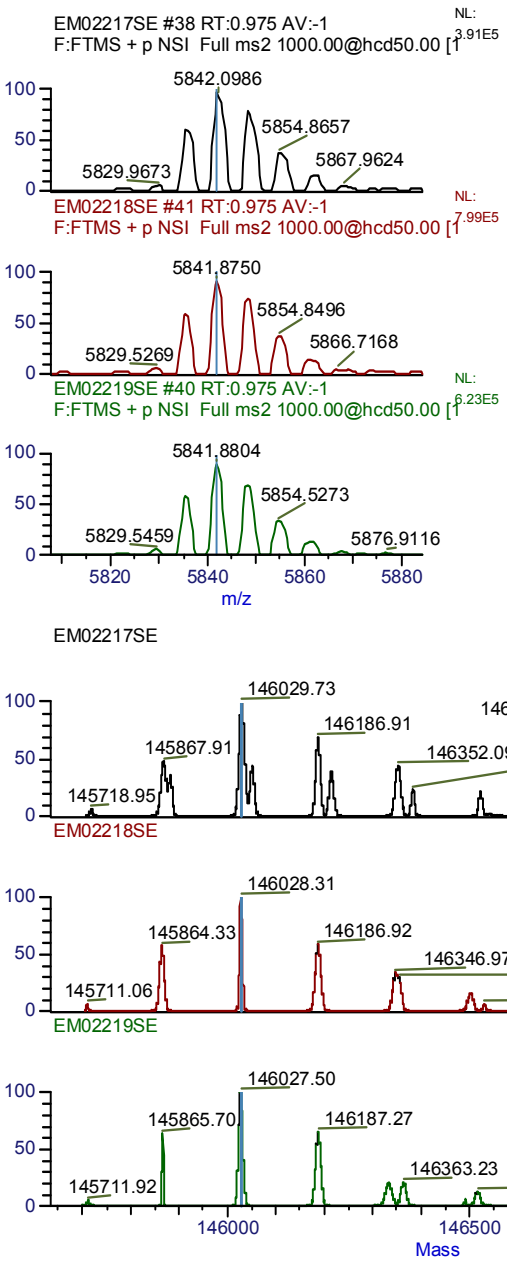
Mass spectra

Trastuzumab in PBS (1x, pH 7.5) + APG (2 equiv.)



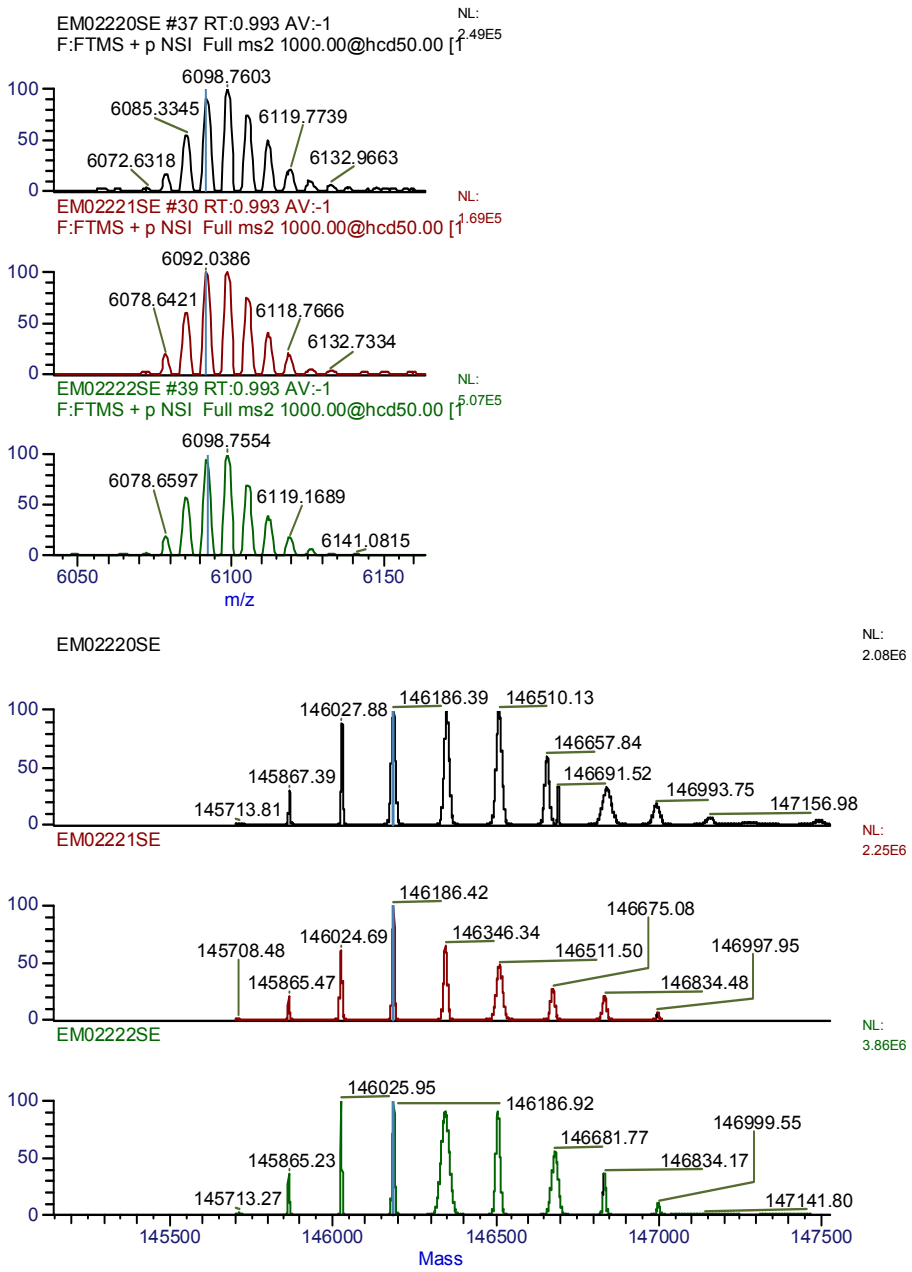
Load	Relative intensity R1	Relative intensity R2	Relative intensity R3	Relative intensity R1(%)	Relative intensity R2(%)	Relative intensity R3(%)	Average R(%)	Std Dev R(%)
0	100,0	100,0	100,0	50	52	49	50	1
1	70,3	67,8	69,9	35	35	34	35	0
2	26,6	21,7	28,4	13	11	14	13	1
3	3,7	3,8	5,0	2	2	2	2	0
DoC	0,7	0,6	0,7					
average DoC	0,67							
Std Dev DoC	0,03							

Trastuzumab in PBS (1x, pH 7.5) + APG (5 equiv.)



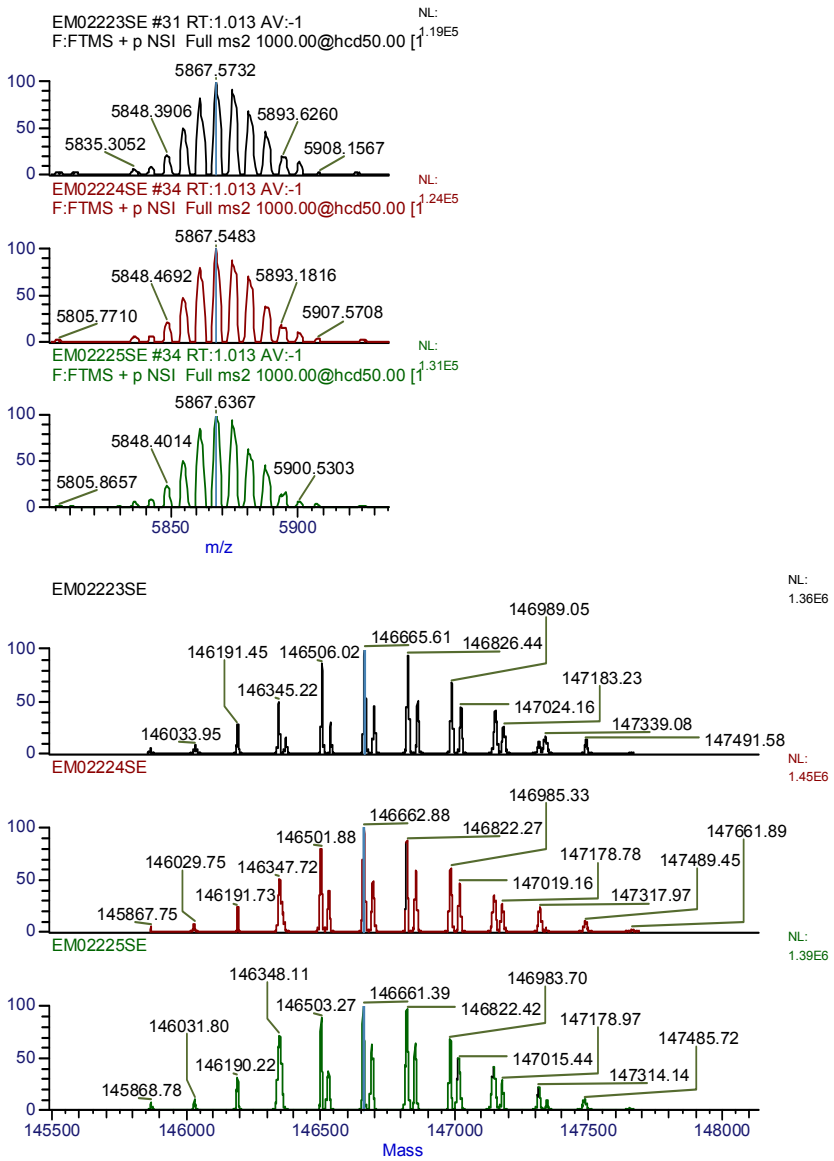
Load	R1	R2	R3	%R1	%R2	%R3	Average %R	Std Dev %R
0	1,70E+06	5,92E+06	5,06E+06	16,48	21,19	23,82	20,50	3,72
1	3,60E+06	1,05E+07	8,11E+06	34,88	37,74	38,13	36,92	1,77
2	2,47E+06	6,06E+06	5,22E+06	23,90	21,72	24,56	23,39	1,49
3	1,56E+06	3,31E+06	1,60E+06	15,07	11,87	7,53	11,49	3,78
4	7,68E+05	1,64E+06	1,03E+06	7,44	5,88	4,85	6,05	1,30
5	2,30E+05	4,49E+05	2,37E+05	2,23	1,61	1,11	1,65	0,56
DoC	1,69	1,48	1,35					
average DoC	1,51							
Std Dev DoC	0,17							

Trastuzumab in PBS (1x, pH 7.5) + APG (10 equiv.)



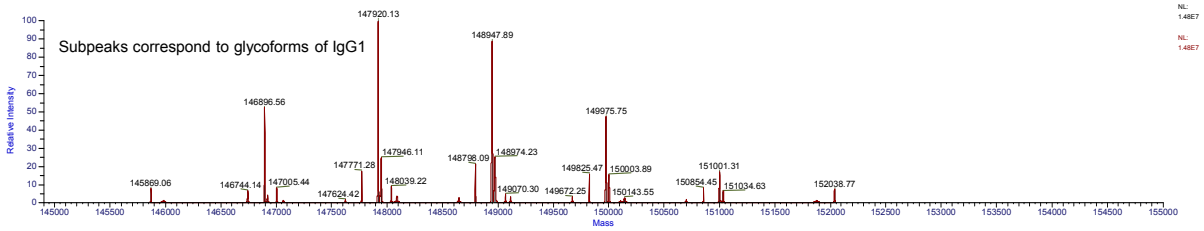
Load	R1	R2	R3	%R1	%R2	%R3	Average %R	Std Dev %R
0	5,99E+05	4,42E+05	1,36E+06	5,45	5,69	6,87	6,01	0,76
1	1,83E+06	1,37E+06	3,86E+06	16,65	17,60	19,47	17,91	1,43
2	2,06E+06	2,25E+06	3,74E+06	18,77	28,97	18,86	22,20	5,86
3	2,05E+06	1,43E+06	3,47E+06	18,67	18,44	17,48	18,19	0,63
4	2,08E+06	1,06E+06	3,49E+06	18,87	13,69	17,60	16,72	2,70
5	1,23E+06	6,04E+05	2,11E+06	11,19	7,78	10,63	9,87	1,83
6	6,48E+05	4,66E+05	1,39E+06	5,89	6,00	7,01	6,30	0,62
7	3,61E+05	1,42E+05	3,75E+05	3,28	1,83	1,89	2,33	0,82
8	1,34E+05	0,00E+00	3,84E+04	1,22	0,00	0,19	0,47	0,65
DoC	3,10	2,73	2,90	100,00	100,00	100,00	100,00	
average DoC	2,91							
Std Dev DoC	0,18							

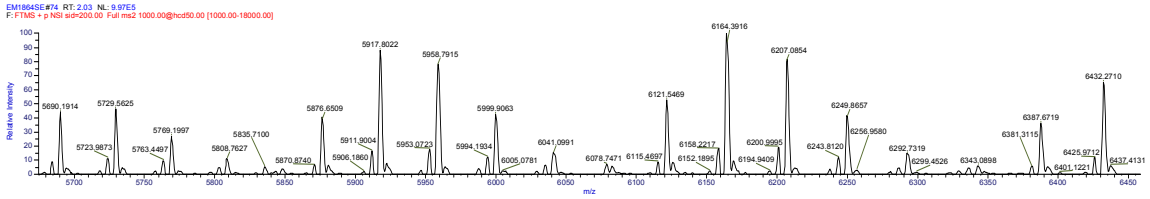
Trastuzumab in PBS (1x, pH 7.5) + APG (20 equiv.)



Load	R1	R2	R3	%R1	%R2	%R3	Average %R	Std Dev %R
0	6,93E+04	7,41E+04	9,02E+04	1,03	1,07	1,20	1,10	0,09
1	9,51E+04	1,04E+05	1,26E+05	1,41	1,50	1,68	1,53	0,14
2	3,65E+05	3,25E+05	4,25E+05	5,41	4,69	5,67	5,26	0,51
3	6,57E+05	6,87E+05	9,89E+05	9,73	9,92	13,20	10,95	1,95
4	1,15E+06	1,14E+06	1,21E+06	17,09	16,50	16,21	16,60	0,45
5	1,36E+06	1,45E+06	1,39E+06	20,13	20,98	18,52	19,88	1,25
6	1,27E+06	1,25E+06	1,32E+06	18,75	18,00	17,59	18,11	0,59
7	9,04E+05	8,72E+05	9,34E+05	13,39	12,59	12,46	12,81	0,50
8	5,57E+05	5,08E+05	5,61E+05	8,26	7,33	7,48	7,69	0,50
9	1,42E+05	3,36E+05	2,91E+05	2,11	4,85	3,88	3,61	1,39
10	1,69E+05	1,50E+05	1,37E+05	2,51	2,17	1,83	2,17	0,34
11	12476,91	2,92E+04	2,00E+04	0,18	0,42	0,27	0,29	0,12
DoC	5,29	5,36	5,19					
average DoC	5,28							
Std Dev DoC	0,09							

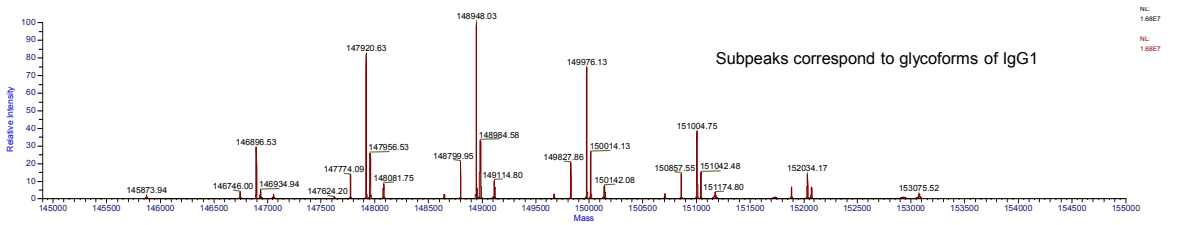
Trastuzumab in PBS (1x, pH 7.5) + APG (8 equiv.) + TAMRA-BCN

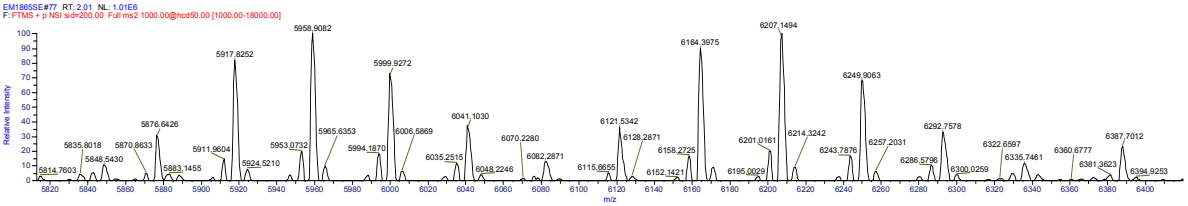




Load	Average mass	Mass Std Dev	Residue mass	Intensity	%R	DoC
0	145869,06	2,90	0	1096605	2	2,61
1	146896,56	3,23	1028	7663698	16	
2	147920,13	2,84	1024	14792338	31	
3	148947,89	4,07	1028	13106295	28	
4	149975,75	3,71	1028	6983681	15	
5	151001,31	3,87	1026	2494513	5	
6	152038,77	4,05	1037	1048885	2	

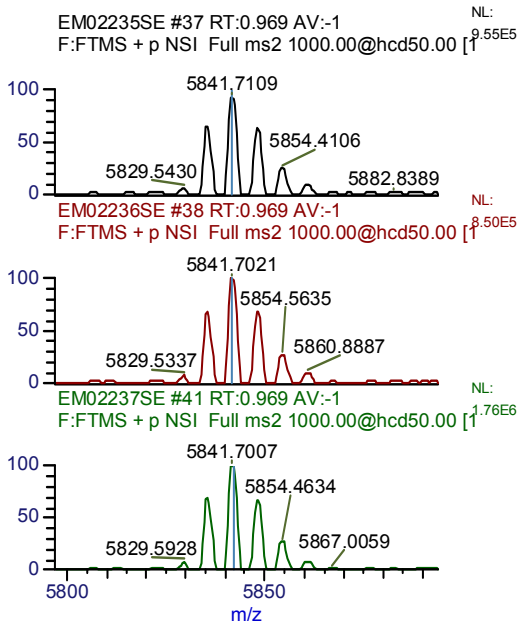
Trastuzumab in PBS (1x, pH 7.5) + APG (11 equiv.) + TAMRA-BCN

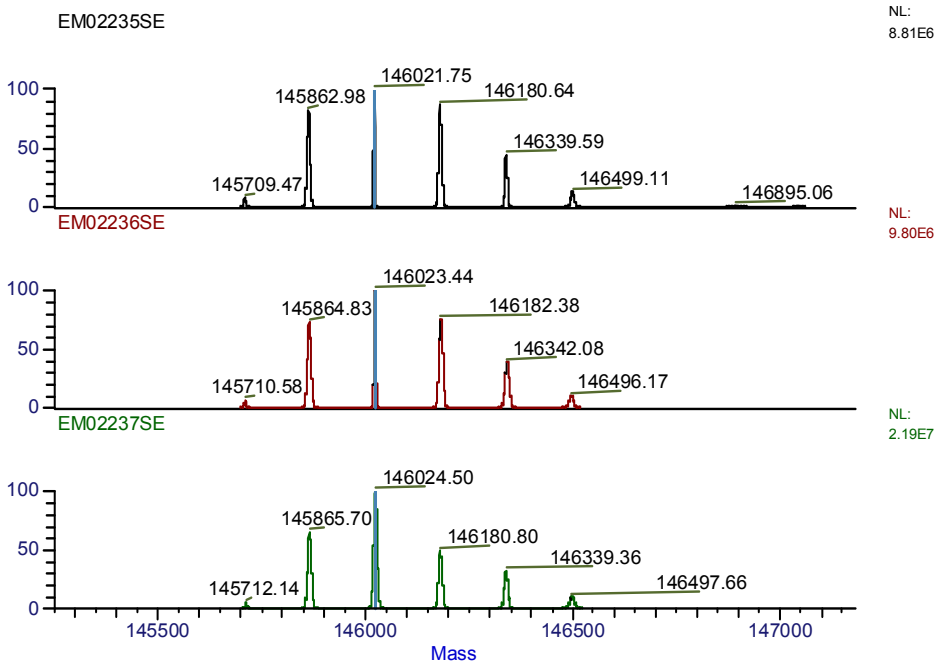




Load	Average mass	Mass Std Dev	Residue mass	Intensity	%R	DoC
0	145869,58	3,81	0	125386	0	3,18
1	146896,53	2,05	1027	4824827	8	
2	147920,63	2,33	1024	13758963	24	
3	148948,03	2,46	1027	16805840	29	
4	149976,13	2,24	1028	12504991	22	
5	151004,75	2,64	1029	6362837	11	
6	152034,17	3,35	1029	2310594	4	
7	153075,52	9,03	1041	438827	1	

Trastuzumab in HEPES/K_2CO_3 (50 mM each, pH 8.7) + APG (2 equiv.)





Load	R1	R2	R3
0	7,16E+06	7,02E+06	1,41E+07
1	8,81E+06	9,80E+06	2,19E+07
2	7,59E+06	7,34E+06	1,07E+07
3	3,83E+06	3,73E+06	6,86E+06
4	1,09E+06	1,00E+06	2,22E+06
DoC	1,40	1,37	1,31
average DoC	1,36		
Std Dev DoC	0,05		
Efficacy, %	68		

References

(1) Dovgan, I., Ursuegui, S., Erb, S., Michel, C., Kolodych, S., Cianférani, S., and Wagner, A. (2017) Acyl Fluorides: Fast, Efficient, and Versatile Lysine-Based Protein Conjugation via Plug-and-Play Strategy. *Bioconjug. Chem. 28*, 1452–1457.

(2) Carapito, C., Lane, L., Benama, M., Opsomer, A., Mouton-Barbosa, E., Garrigues, L., Gonzalez de Peredo, A., Burel, A., Bruley, C., Gateau, A., Bouyssié, D., Jaquinod, M., Cianferani, S., Burlet-Schiltz, O., Van Dorsselaer, A., Garin, J., and Vandenbrouck, Y. (2015) Computational and Mass-Spectrometry-Based Workflow for the Discovery and Validation of Missing Human Proteins: Application to Chromosomes 2 and 14. *J. Proteome Res. 14*, 3621–3634.