Electronic Supplementary Material (ESI):

1.1 Protein determination

The concentrations of tagged proteins were determined by a micro plate spectrophotometer Spectramax Plus 384 (Molecular Devices, Sunnyvale, USA) using the Bradford assay. External calibration was carried out at an absorption maximum at 595 nm by using a dilution series of bovine serum albumin (Sigma Aldrich, Taufkirchen, Germany).

1.2 Antibody tagging via MeCAT (metal coded tag)

A detailed description of the of antibody tagging procedure is given in reference [1]. For our investigation we have chosen MeCAT (Ho) (Proteome Factory AG, Berlin) for tagging of anti-Her 2 antibody. Summary, the tagging method started with a partial reduction of the antibody with a molar excess rate of 600 of TCEP relative to antibody molarity for 30 min at 37°C. After purification by ultra-filtration at 7500 x g of the partial reduced antibody the modification was made using a 20 fold molar excess of MeCAT (Ho) (Proteome Factory AG, Berlin) which was agitated for 60 min at 37°C. After reaction the MeCAT excess was removed from the antibody solution by ultra-filtration as described above.

1.3 Immunohistochemistry (IHC) staining of FFPE breast cancer tissue sections for LA-ICP-MS detection

The human epidermal growth factor receptor 2 (Her-2) is an important tumour marker, which is detected in the clinical routine diagnostic in breast cancer tissue section with IHC staining.
FFPE breast cancer tissue and normal breast tissue were sectioned using a commercial sliding microtome (HM355S, Microm International GmbH, Walldorf, Germany) with a thickness of 5 µm for an IHC staining and subsequent LA-ICP-MS measurement. All sections were immobilized onto Superfrost Plus slides (Thermo Fisher Scientific, Braunschweig, Germany) and deparaffinised in xylene, followed by a series of graded alcohols. All tissue sections were pretreated with a target retrieval solution pH 6 (S1700, Dako) for 20 min at 90°C. After cooling to room temperature, the tissue was washed with a TBS / Tween buffer (S3306, Dako) for 5 min. To block endogenous peroxidase, the tissues were incubated with the Dako Real TM peroxidase-blocking solution (Dako EnVisionTM Kit) for 10 minutes by room temperature. This was followed by repeating washing steps for 2 x 5 minutes with TBS/Tween buffer.

In this work, anti- Her-2 antibody was tagged via MeCAT (Ho) as described in ESI section 1.2. The holmium tagged antibody was diluted with Dako Real TM Antibody Diluent (S2022, DAKO). The tissue section was incubated with a tagged antibody for 1 h by 37°C in an incubator with a concentration25 of 1 μg mL^{-1}. In order to prevent the drying of the tissue section, the incubation was performed in a hybridization chamber. This was followed by two washing steps with TBS/Tween-washing buffer for 5 minutes each. Subsequently treatment with the secondary antibody (Dako REAL TM EnVision TM HRP Rabbit/Mouse) was carried out for 30 min at room temperature. After incubation the unbound antibody was removed by washing steps with buffer as described above. Visualization was performed by incubation with a 3,3`diaminobenzidine (DAB) substrate-chromogen solution (Dako REALTM EnVisionTM HRP Rabbit/Mouse) for 10 min according to the manufacturer of the kit resulting in brownish coloring. For laser ablation, the tissue was dehydrated through a graded alcohol series consisting of 70%, 90% and 100% ethanol according to conventional IHC protocols.

1.4 Pre-treatment of mouse tissue sections for LA-ICP-MS detection

In a pretest formalin-fixed mouse brain tissue section was incubated with an MeCAT loaded erbium tagged antibody against an “house-keeping protein” called GAPDH. The tissue section was deparaffinized in Neo-Clear (alternative xylene) for 3 x 5 min and rehydrated through a series of alcohols (100%, 96%, 70%) for 2 x per 30 sec. After rehydration the tissue section was transferred into distilled water. For the unmasking of antigens the tissue section a 10 mM citrate buffer (PH 6-6.8) was used. The buffer was heated for 2 min at 650 watt in a microwave. Subsequently, the tissue was boiled in the warm buffer for 2 x 5 min in the microwave (650 watt). With the aid of ice the tissue section with the citrate buffer was cooled. Thereafter, the tissue section was incubated with a 0.3 % peroxidase blocking solution for 5 min. This was followed by three brief wash steps with PBS buffer (12 mM phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4). The MeCAT (Er) modified antibody was 1:50 or 1:300 (depending on the antibody) with BSA PBS buffer diluted. The tissue section was incubated with 250 μL of the modified antibody for 1 h at room temperature in a hybridization chamber. In the following step the tissue section was rinsed with 12 mM PBS buffer (137 mM NaCl, 2.7 mM KCl, pH 7.4) to remove unbound antibodies, and dehydrated through graded alcohols (70%, 96%, 100%) prior to laser ablation.

1.5 Iodination

Iodination of tissues sections was performed with a saturated KI3 solution in accordance with reference.2 After termination of the reaction with sodium dithionite (Merck, Darmstadt, Germany) and rinsing with PBS-T (phosphate buffered saline pH 7.3: 137 mM NaCl, 2.7 mM KCl,4.3 mM Na2HPO4, KH2PO4 (all from Merck, Darmstadt, Germany) the tissue sections were dried in the air.

Table 1 Summary of the acquisition parameters for LA-ICP-MS.

ICP-MS (Element XR)	
RF plasmasource power	1350 W
Plasma gas flow	15 L min^{-1} Ar
Auxiliary gas flow	0.8 L min^{-1} Ar
Transport gas flow	0.8 L min^{-1} Ar
Mass resolution (*m*/Δ*m*)	300
Scanning mode	E scan
Sample time	2 ms
LA system (New Wave 213)	
Wavelength	213 nm
Helium gas flow	1 L min^{-1}
Laser energy	0.20 - 0.25 mJ (35%)
Laser spot size	200; 100;50 µm
Scan speed	200; 100; 50 µm s^{-1}
Overlap of the laser spot size	180; 80; 30 µm
Repetition frequency	20 Hz
Ablation mode	Scanning line per line
Analysis time per slice	1 – 2 h

Table 2 Summary of the calculated values (3.0-7.6 mm). Laser parameters: laser spot size 50 µm, scan speed 50 µm s^{-1}, resolution of 30 µm.

	^{165}**Ho**	^{165}**Ho/**^{127}**I**	^{165}**Ho/**^{115}**In**
signal-to-background ratio (SBR)	18	11	39

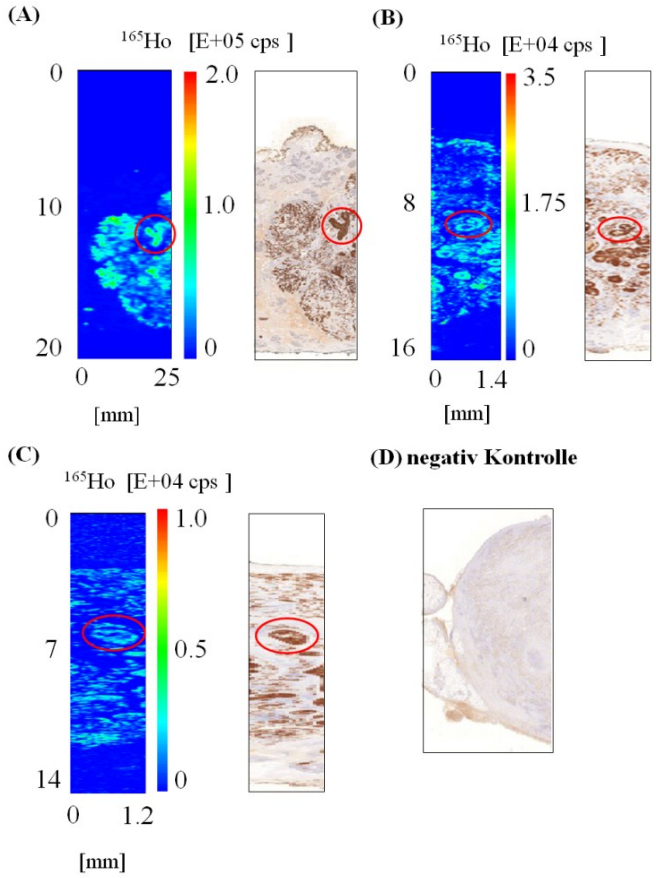

Fig. 1 LA-ICP-MS images and corresponding immunohistochemical (IHC) staining of a 5 µm breast cancer tissue section. Positive areas for anti-Her-2 antibody are shown in (A-C), while (D) shows a negative control for Her-2 in comparison. Images resulting from LA-ICP-MS acquisition using different laser parameters are pointed out in part (A), (B) and (C) with their IHC counterparts. The individual positive tumour areas are marked with red circles. Laser parameters: (A) laser spot size 200 µm, scan speed 200 µm s^{-1}, overlap of the laser spot size of 180 µm, (B) laser spot size 100 µm, scan speed 100 µm s^{-1}, overlap of the laser spot size of 80 µm, (C) laser spot size 50 µm, scan speed 50 µm s^{-1}, overlap of the laser spot size of 30 µm, repetitions rate 20 Hz, laser energy 0.20 - 0.25 mJ (35%).