Mini-Protean Tgx, Bio-Rad Laboratories, Switzerland). The gel was stained with Coomassie Blue G-250 (Bio-Safe Coomassie Stain, Bio-Rad Laboratories, Switzerland). The visualized gel bands were then cut into 5 slices per lane and further cut up into smaller pieces. The In-gel digestion was done according to Shevchenko et al. (2006). Briefly, the gel pieces were dehydrated and washed. Disulfide bonds were reduced with 10 mM dithiothreitol, followed by an alkylation step using 55 mM iodoacetamide. The samples were digested with trypsin (Sequencing Grade modified trypsin, Promega, Switzerland) at 37°C overnight. The resulting peptides were extracted using 100 µL of 5% formic acid/ acetonitrile (1:2, v/v) followed by a second extraction step using 20 µL 1% formic acid/ acetonitrile (1:1, v/v). Both supernatants were collected into a 250 µL vial and dried to dryness with a vacuum centrifuge (GeneVac EZ-2 Plus, SP Scientific, UK). The peptide mixture was re-dissolved with 30 µL of 0.1% formic acid and analysed by liquid chromatography coupled to a tandem mass spectrometer.

LC-MS/MS analysis

An Agilent 1290 Infinity LC system connected to an Agilent 6540 quadrupole time-of-flight mass spectrometer (Agilent Technologies, Switzerland) was used. The peptide mixtures (12 µL) were injected onto a reversed phase C18 column (2.1 x 150 mm, 2.7 micron, 120 Å pore size, Agilent Technologies, Switzerland). The column flow was set to 400 µL/min with a mobile phase composed of solvent A (water) and B (acetonitrile with 0.1% formic acid). Initially, 2.5% of mobile phase B was held constant for 1 min, followed by a linear gradient from 2.5% – 35% B over 90 min, and a final increase over 2 minutes to 95% B where it was held for 2 min. Re-equilibration of the initial column condition was performed within 6 minutes. Column temperature was held constant at 45°C through the run. The jet stream electrospray source was operated in positive mode with following parameter settings: nebulizer pressure 35 psig, nozzle voltage 0 V, sheath gas flow 12 L/min, sheath gas temperature 250°C, drying gas flow 10 L/min, drying gas temperature 250°C, capillary voltage 4000 V and fragmentor

voltage of 175 V. The instrument state mass range was set to 1700 m/z with 4 GHz digitalization rate, which leads to a mass resolution of 40 000 (measured at m/z 1521). Mass spectra were acquired in profile mode over a m/z range of 100 - 1700 by 1 Hz in scan and 3 Hz in MS/MS mode. The auto-MS/MS experiment was selected using the 20 most abundant precursor ions per cycle with activated precursor abundance-based scan speed. The quadrupole precursor ion isolation width was set to medium (~ 4 m/z) with a dynamic collision energy based as a function of the m/z value (slop = 3, offset = 2). Active exclusion of a precursor mass was one repeat and release after 15 seconds. The system was controlled with MassHunter Data Acquisition B.08.01 service pack 1 (Agilent Technologies, USA).

Protein identification

The raw data files were processed with Mascot Distiller 2.7 (Matrix Science, UK) to generate a peak list. A database search to identify proteins was conducted using an in-house Mascot Server 2.6 (Matrix Science, UK) against the *Apis mellifera* sequence database (15323 protein sequences of *Apis mellifera*, downloaded in November 2018 from Uniprot.org) and sequences for common protein contaminants (cRAP, downloaded from thegpm.org). The search parameters were: enzyme trypsin, maximum missed cleavages 2, fixed modification carbamidomethylation of cysteine and variable modification oxidation of methionine. Peptide mass tolerance window was set to 7 ppm and fragment ion mass tolerance to 0.70 Da. The received search results were sent to the Scaffold software 4.7.3 (Proteome Software, USA). The loaded results were searched against a second search engine X! Tandem (thegpm.org, version Cyclon 2010.12.01.1) to increase confidence. The Local FDR algorithm with 95 % probability was used for validating peptide identifications. The Protein Prophet algorithm with 99.0 % probability was used for protein identification, and contained at least 2 identified peptides (Nesvizhskii et al. 2003).