

NMR quadriceps sample preparation protocol

For ^1H -NMR spectroscopy, white quadriceps muscle extracts (70 μL) were transferred to glass vials (2 mL, Agilent) and evaporated under a stream of nitrogen gas at 37 °C. On the day of analysis, each dried extract was resuspended in sterile, filtered water (70 μL) to achieve single signal suppression (NOSEY pre-saturation) and centrifuged at 12 000 $\times g$ (5 min, 25 °C) to remove macromolecules and other particulates. A buffer solution (pH 7.4) was prepared as described by Dona *et al.* (2014) containing 1.5 M KH_2PO_4 dissolved in D_2O together with 100 ppm trimethylsilyl-2,2,3,3-tetradeuteriopropionic acid (TSP) as chemical shift standard and 13 ppm NaN_3 . Using an eVol® NMR digital syringe, the sample ultra-filtrate (54 μL) together with 10% buffer solution (6 μL) was aspirated and the total volume (60 μL) was purged into a 2 mm NMR tube (outside \varnothing 2.0 mm, inside \varnothing 1.6 mm, length 100 mm). The sample was then mixed once inside the 2 mm NMR tube to ensure homogeneity by aspirating and purging the entire volume. Thereafter, a wash sequence was used to clean the syringe before the next sample was prepared: (1) aspirate 100 μL distilled water, (2) purge 100 μL (waste), (3) aspirate 100 μL distilled water, (4) purge 100 μL (waste), (5) aspirate 100 μL distilled water, (6) purge 100 μL (waste). Next, the NMR tubes were assembled using the Bruker MATCH system, which is an adapter with a gripper to hold the 2 mm NMR tube, inserted into a 10 mm spinner. Finally, each NMR MATCH assembly was loaded onto a SampleXpress autosampler for NMR analysis.