Collection of NMR Spectra of EBC

To maximize the sensitivity and resolution of NMR measurements of inherently dilute EBC specimens, we collected their spectra with a Bruker Avance III 800 MHz spectrometer with 5 mm TCl cryogenic probe. EBC samples were prepared with 7% D_2O and 20 μ M trimethylsilyl propanoic acid as 0 ppm reference standard. 1D ¹H NMR spectra were collected using W5-WATERGATE water suppression [31] supplemented by presaturation of the water resonance at minimal power. 32768 transients were normally averaged. After data collection, the ¹H NMR free induction decays were zero filled to 32,768 points, apodized with 1.3 Hz exponential broadening, and Fourier transformed into spectra spanning from -2 to 12 ppm, with correction of phases and baseline using Bruker Topspin 3.1.

Spectral Preprocessing

About 30,000 points from 0.02 to 10 pm were retained for analyses, while the region around the suppressed water peak from 4.5 to 5.38 ppm was omitted. To accommodate the large variability in overall concentrations of biomolecules condensed.