Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information

Water Dynamics in Human Cancer and Non-Cancer Tissues

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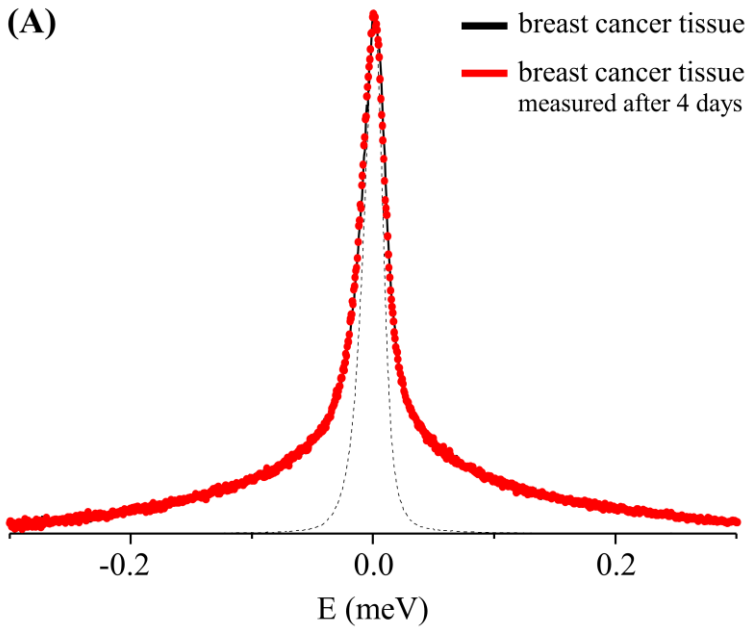

Figure S1. QENS profiles (for all Q values, at 310 K) for human breast cancer tissue, measured with a 4-day time lapse (during which the sample was stored at -80 ºC). (The QENS profiles were normalised to the maximum peak intensity; the dashed line represents the instrument resolution, as measured by a standard vanadium sample).

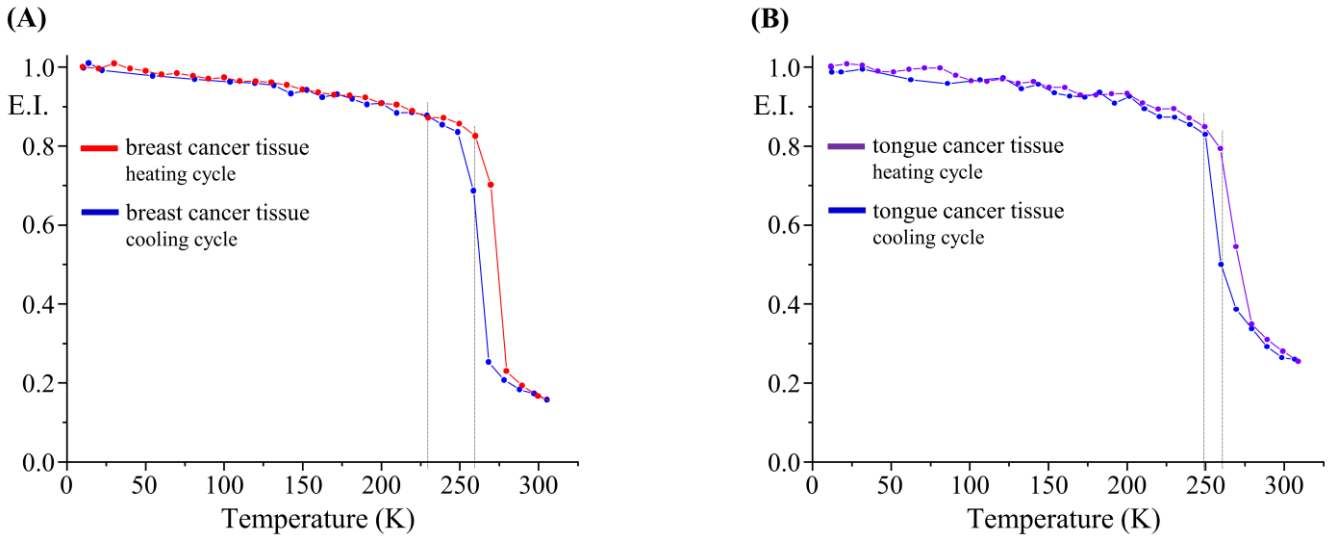

Figure S2. Elastic scan plots as a function of T (10-310 K) for human breast and tongue tissues cooling and heating cycles: (A) breast cancer; (B) tongue cancer. (The plots represent the elastic intensity integrated over the IRIS instrumental resolution, normalised to the intensity obtained at the lowest temperature (10 K)).

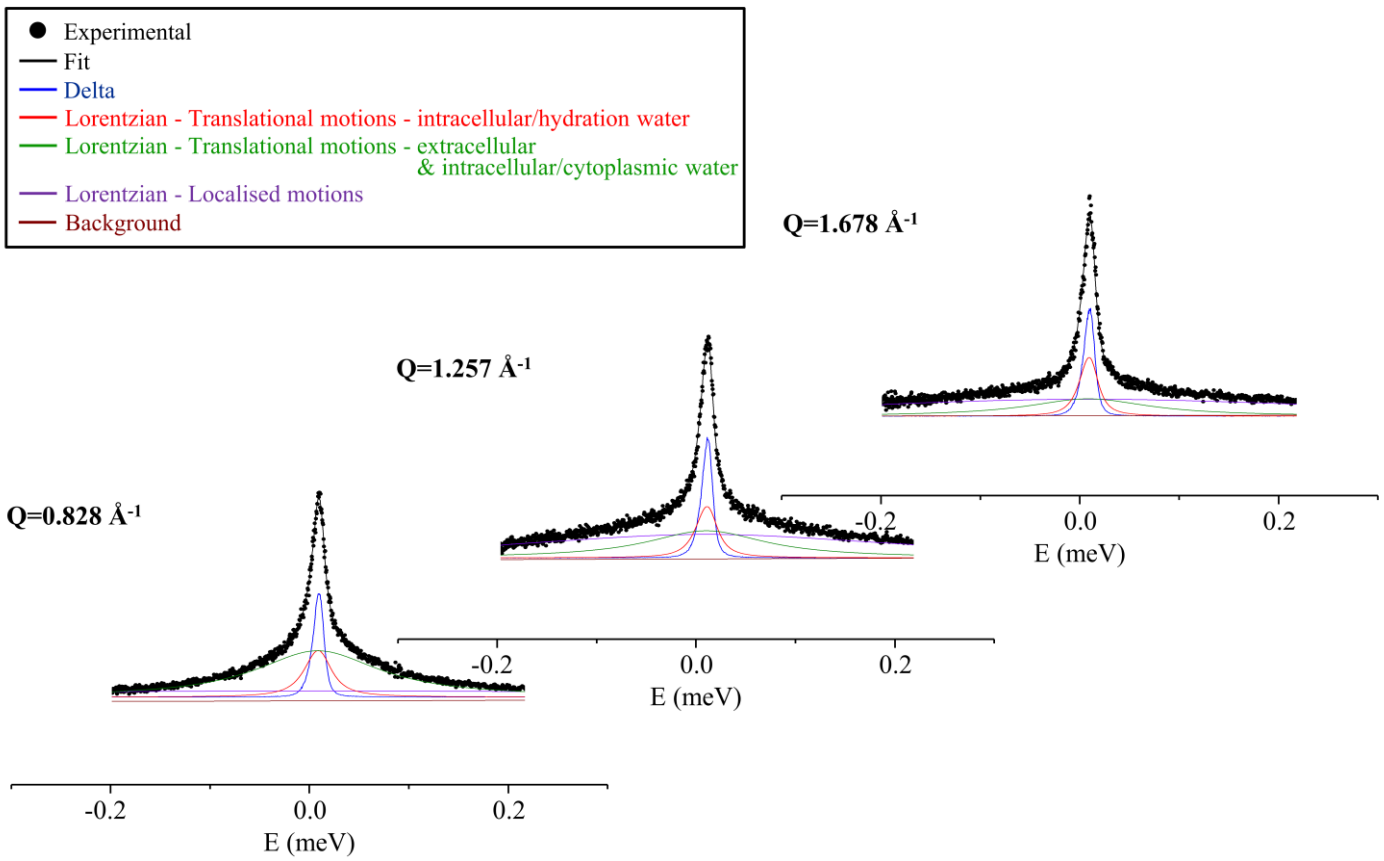

Figure S3. QENS profiles (at 310 K, normalised to the maximum peak intensity) for human breast cancer tissue, fitted using three Lorentzians and one Delta functions, at some typical Q values.

Experimental

Chemicals

Optimum Cutting Temperature (OCT) embedding medium was purchased from Thermo Fisher Scientific (MA, USA).

QENS Fundamentals and Measurements

Quasi-elastic incoherent neutron scattering (QENS) typically analyses the incoherent scattering signal resulting from a variety of atomic motions ranging from fast vibrational and rotational localised modes, to slower diffusional modes, motions taking place at pico- to nanosecond timescales, and exploits the high sensitivity of neutrons to hydrogen (with a much larger incoherent scattering cross section compared to that of other elements). The QENS signal, the so-called dynamic structure factor, S(Q,ω), is due to a variety of dynamical processes (which fall in the spectrometer's time window), from fast localised modes including vibrations and rotations to slower global translational motions. It arises from energy (ħω) and momentum (Q) exchanges between the neutrons and the atoms within a given spectrometer resolution, and is detected as a broadening about an elastic line of energy exchange ≈0. The measured signal can be mathematically expressed as:

$$S_{measured}(Q,\omega)=\exp\left(-\frac{\hbar\omega}{2kT}\right)R(Q,\omega)\otimes S(Q,\omega) \quad (1)$$

where exp[-ħω/2kT)] is a detailed balance parameter, and R(Q,ω) is the instrument´s resolution function (experimentally obtained) which is convoluted with the scattering model (S(Q,ω)) that describes the dynamical behaviour of the sample. In hydrogenous biological samples, S(Q,ω) is dominated by the incoherent scattering of the hydrogen atoms (much larger than the coherent or incoherent scattering cross section of any other atom) and are approximated as the convolution of vibrational, rotational and translational components (assumed as independent motions),

$$S_{inc}(Q,\omega)=S_{vib}(Q,\omega)\otimes S_{rot}(Q,\omega)\otimes S_{trans}(Q,\omega) \quad (2)$$

Strictly in the elastic and quasielastic regions,

$$S_{inc}(Q,\omega)=\exp(-Q^2\langle u^2\rangle)\left[A_0(Q)\delta(\omega)+\left(1-A_0(Q)\right)L(Q,\omega)\right] \quad (3)$$

where the exponential term is the Debye-Waller factor, $A_0(Q)\delta(\omega)$ is the elastic contribution due to motions slower than the longest observable time as defined by the energy resolution of the spectrometer, and the second term in the equation corresponds to the quasielastic component. Sinc(Q,ω) provides time/space information on the system probed, on the timescale of the dynamical processes (through the neutron energy transfer, ω), and on the spatial extent of these processes (from the momentum scattering transfer, Q). Once the QENS data in the time-domain is represented by an exponential, it can be approximated in the energy domain by Lorentzian functions of different widths,

$$L(x)=\frac{1}{\pi}\frac{\Gamma}{\Gamma^2+\omega^2} \quad (4)$$

Γ being the full width at half-maximum (FWHM=2xHWHM (half-width at half-maximum)). These Lorentzian functions describe motions occurring on different timescales, detailed information on each dynamic component being retrieved from the Q-dependency of Γ.

The QENS experiments were performed at the ISIS Pulsed Neutron and Muon Source of the Rutherford Appleton Laboratory (http://www.isis.stfc.ac.uk/), in the low-energy backscattering spectrometer IRIS,[1,2] using the 002 reflection of the cooled pyrolytic graphite analyser bank (PG002 50Hz configuration) with a 17.5 μeV energy resolution (FWHM). The data was recorded in the Q range 0.42 to 1.85 $Å^{-1}$ (covering a 25º to 160º angular interval, for a total of 51 detectors/Zn scintillators).

The tissue samples (*ca.* 150-200 μl), as well as OCT, were loaded into indium-sealed flat Al cans (3x5 cm, 0.1 mm thickness), for a 2.2x4.4 cm beam size at the sample. The thickness of the tissue sections was chosen in order to reduce neutron absorption from H_2O and circumvent multiple scattering events. The samples were oriented at -30º with respect to the incident beam. The water sample was loaded into an indium-sealed annular Al can.

Measurements were performed at both 298 and 310 K (physiological temperature). Elastic window scans (10 – 310 K) were also obtained for all samples. For each sample, the QENS acquisition (at 310 K) was followed by a resolution function (at 10 K) and an elastic scan (10 – 310 K). Acquisition times were 8 to 10 h for the QENS measurements (1500 to 2000 μA) and 6 to 7 h for the elastic scans (1200 μA). A vanadium sample (a purely incoherent elastic scatterer) was measured to define the instrument resolution and correct for detector efficiency.

QENS Data Analysis

The quasi-elastic neutron scattering data was grouped and reduced from raw time-of-flight signals into energy transfer spectra using the MANTID routine (version 6.1). [3]

QENS spectra were corrected for detector efficiency. Resolution functions were determined independently from calibration runs for: (i) vanadium; (ii) breast cancer and normal tissues; (iii) tongue cancer and normal tissues.

Fitting of the QENS spectra was performed for the energy transfer range -0.3 to 1.2 meV, with the DAVE software (version 2.5, developed at the National Institute of Standards and Technology (NIST) Center for Neutron Research).[4] The tissue systems were accurately represented by applying one Dirac Delta function to account for very slow hydrogen motions whose center of mass appears immobile within the instrument resolution (elastic component) and three Lorentzians that describe all mobile atoms within the instrument´s timescale (quasielastic contributions). In addition, an energy independent instrumental background was applied.

FWHM values were extracted from each of the Lorentzian functions, and the translational diffusion coefficients (D_T) and reorientation times (τ_T, the mean residence time of a water molecule in each possible location) (at temperature T) were obtained according to a non-diffusive jump reorientation model [5-7] that follows the equation:

$$\Gamma_T(Q) = \frac{D_T Q^2}{1 + D_T Q^2 \tau_T} \qquad (5)$$

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