Supplementary Information

Dynamics of heparan sulphate explored by neutron scattering

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Sample preparation for neutron scattering

Porcine intestinal mucosa heparan sulfate (HS) was purchased as a lyophilised powder from Celsus laboratories (Cincinnati, Ohio). The average molecular weight of the sample is 12 kDa with a polydispersity of 1.59, determined by B. Mulloy (as described in Mulloy et al.[1]). The sulphation degree was evaluated by elemental analysis. S and N contents were found to be 6.96 and 2.15 % respectively, which corresponds to about 1.4 sulphate groups per disaccharide in average. The sample features highly N-sulphated (NS) domains interspersed with N-acetylated (NA) domains as demonstrated by the sensibility of the molecule to both heparinase and heparitinase (data not shown). Note that only NA domains contain methyl groups, with less than one methyl group per NA disaccharide in average. The charges of the sulphate groups are neutralised by the charges of the sodium ions also present in the powder.

The powder was three times taken up in D_2O and then lyophilised. Three samples (dry, partially hydrated and hydrated) were prepared for the neutron scattering measurements. HS powder was placed on three 4 x 3 cm^2 flat aluminium sample holders, and dried over silica gel for one day to remove the remaining water molecules. HS weights corresponded to 166, 202 and 148 mg for the three samples cited above, respectively. The weights were used to define the hydration level of 0 g of D_2O per g of HS. The first sample was sealed in the dry state, and called 'HS-dry'. The two others were hydrated over vapour pressure of D_2O, until the weight reached 0.15 g D_2O per g of HS (denoted HS-0.15g/g) and 0.43 g D_2O per g of HS (denoted HS-0.43g/g), respectively. The samples were sealed with indium and closed with aluminium covers to give a sample chamber of 0.4 mm thickness. HS-0.15g/g contained 31 mg D_2O,

equivalent to a hydration level of 0.14 g H_2O / g HS. HS-0.43g/g contained 63 mg D_2O, corresponding to 0.39 g H_2O / g HS.

Neutron scattering experiments

Experiments were performed on the time-of-flight spectrometer IN6 and the backscattering spectrometers IN13 and IN16 at the Institut Laue-Langevin (ILL), Grenoble. Spectrometer characteristics are provided in Table 1. All samples were measured on each spectrometer, except for HS-dry that was not measured on IN16. The samples were placed in a cryostat or a cryofurnace at room temperature at an angle of 135º with respect to the incident neutron beam, and then cooled to 20 K in a few hours. The measurements were performed on heating at given temperatures from 20 K to 300 K. On IN13 and IN16 elastically scattered neutrons were recorded; on IN6 the full energy-range was recorded and the elastic intensity was extracted by integrating over 10 channels in a time window around the elastic peak. Data were analysed using the LAMP program and implemented routines[2]. The signal from the empty sample holder was subtracted and data were normalised to the intensity at 20 K that also corrects for detector efficiency. The atomic mean square displacement (MSD), $< u^2 >$, of motions that are localised within the time- and length- window of the spectrometer was calculated from the Q-dependence of the elastic intensity according to the Gaussian approximation[3]:

$$I_{el}(Q,\omega=0) = I_{el}(0)\exp(-\frac{1}{6} < u^2 > Q^2) \quad (1)$$

I_{el} is the normalised elastically-scattered intensity and $I_{el}(0)$ the value of the intensity at $Q = 0$. The approximation is valid for $Q^2 < u^2 > < 2$.

Table 1. Spectrometer characteristics

Spectrometer	Wavelength (Å)	Measured Q-range ($Å^{-1}$)	Associated length-scale $1/Q$ (Å)	Energy resolution (μeV)	Associated time-scale up to
IN6	5.12	0.4 – 2.1	0.5 – 2.5	90	10 ps
IN13	2.23	0.5 – 2.3	0.4 – 2	8	100 ps
IN16	6.27	0.5 – 1.9	0.5 – 2	0.9	1 ns

Elastic data

The normalised elastically-scattered intensity obtained for HS-0.43g/g is presented in Figure S1. MSD for all samples were extracted in the ranges $0.4 < Q^2 < 2.1$ Å^{-2} from IN6 data, $0.5 < Q^2 < 2.3$ Å^{-2} from IN13 data, and $0.5 < Q^2 < 1.9$ Å^{-2} from IN16 data.

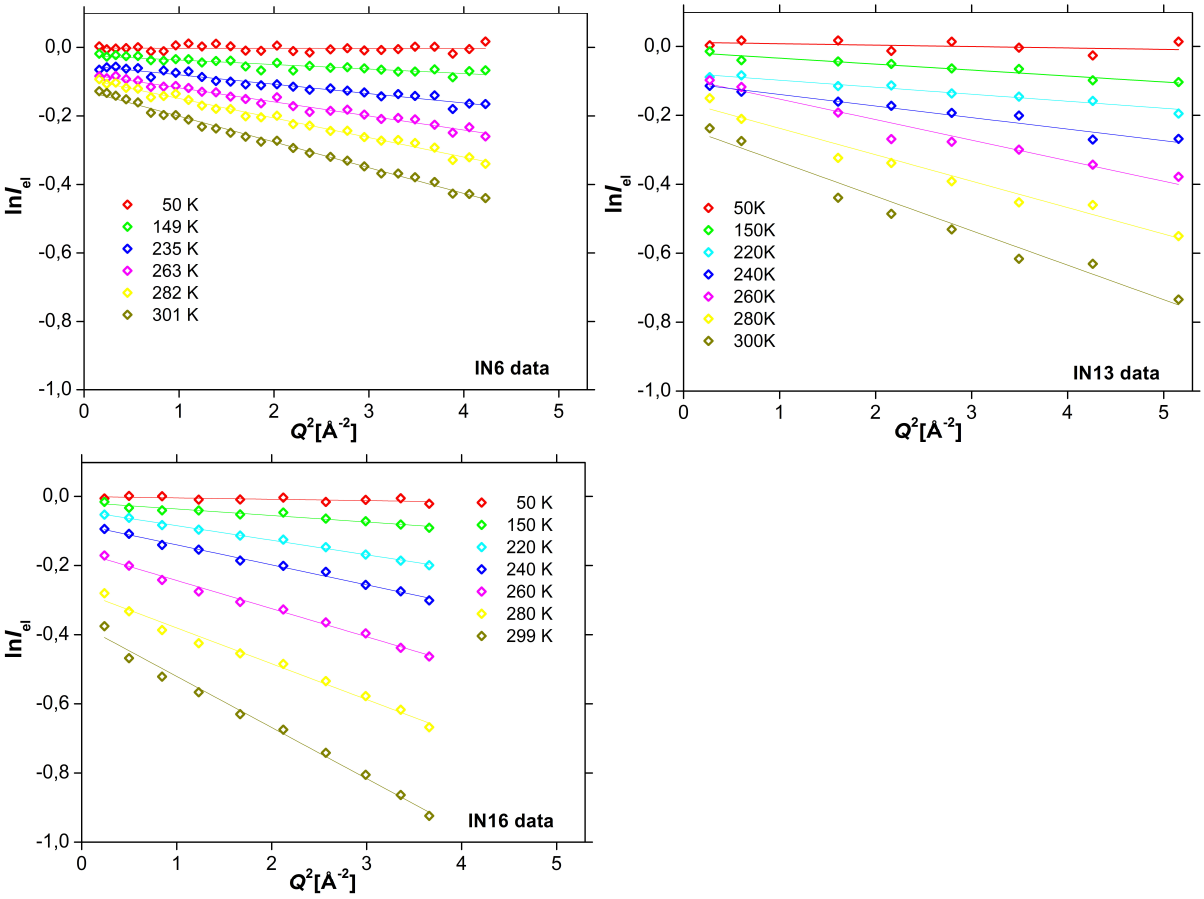

Figure S1. Logarithm of the normalised elastic intensity from HS-0.43g/g sample measured on IN6 (*top-left*), IN13 (*top-right*) and IN16 (*bottom-left*) at different temperatures with corresponding linear fits.

Mean square displacement for HS-dry

Figure S2 reports the temperature dependence of the MSD for the HS-dry sample on the ps (IN6) and 100 ps (IN13) time-scales. MSD on the two time-scales examined are the same and remain harmonic as a function of temperature. The identical MSD values found for both data sets revealed that the two spectrometers measure the same movements, which occur on the faster, ps, time-scale.

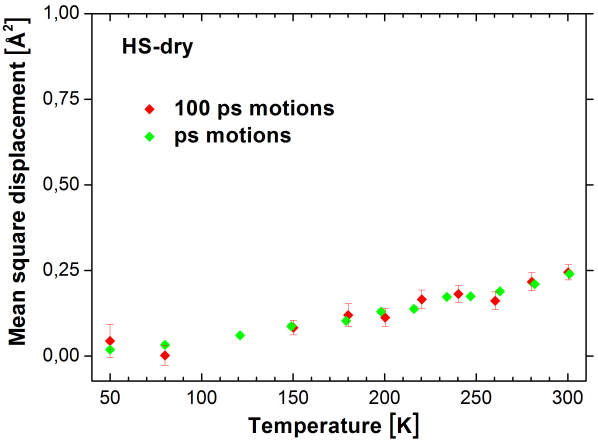

Figure S2. Mean square displacements (MSD) extracted for HS-dry on the ps (IN6) and 100 ps (IN13) time-scales.

References

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