- $1 \quad \text{Complexity of dissolved organic matter in the molecular size dimension: insights from coupled size}$
- 2 exclusion chromatography electrospray ionisation mass spectrometry
- **3** Supplementary Information
- 4 J. A. Hawkes, P. J. R. Sjöberg, J. Bergquist and L. J. Tranvik
- 5



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- 7 Figure SI1: Effect of changing injection volume (left) and concentration (right). Injection volumes
- 8 between 1-20 μL at equal loading amounts had little effect on the UV retention profile (see
- 9 normalized signal in lower panels), and increasing sample concentration at 10 µL injection also had
- 10 no effect on the normalized profile.



- 12 Figure SI2: Effect of in-source CID on extracted ion current in three mass ranges. Black = 0 V, Red = 30
- 13 V, Green = 60 V, Blue = 90 V. In-source CID would be expected to break weak aggregates already at
- 14 low voltages, leading to an increase in m/z 200-400 between 7-8 minutes if the signal from m/z 800-
- 15 2000 is composed of aggregated monomers of low masses. This effect was not observed.



- 17 Figure SI3: Total assigned current (TAC) (black dots) and UV response (blue dots) in 74 headwater
- 18 streams after sample pre-concentration based on DOC concentration. UV response is relatively
- 19 similar across the sample set, whereas TAC decreases significantly as DOC increases. This indicates
- 20 that a large portion of material was not characterised by MS in this study (Hawkes et al. 2018),
- 21 probably high molecular weight phenolic material that is more predominant in DOC rich streams.



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- 23 Figure SI4: Microspecies distribution for the three model compounds at pH 6.6. The Malonic acid
- 24 derivative is mainly the 4-charge species, with an important amount of 3-charge. The Isoferulic acid
- 25 glucuronide is entirely the 2-charge species, while Fraxin is mainly uncharged, but has some small
- 26 proportion <10% with a charged alcohol group. Figure made in Marvin Sketch.