separations

Appendix A for the publication:

Article

Living with Breakthrough: Two-Dimensional Liquid-Chromatography Separations of a Water-Soluble Synthetically Grafted Bio-Polymer

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Appendix A

S1 Fractionation

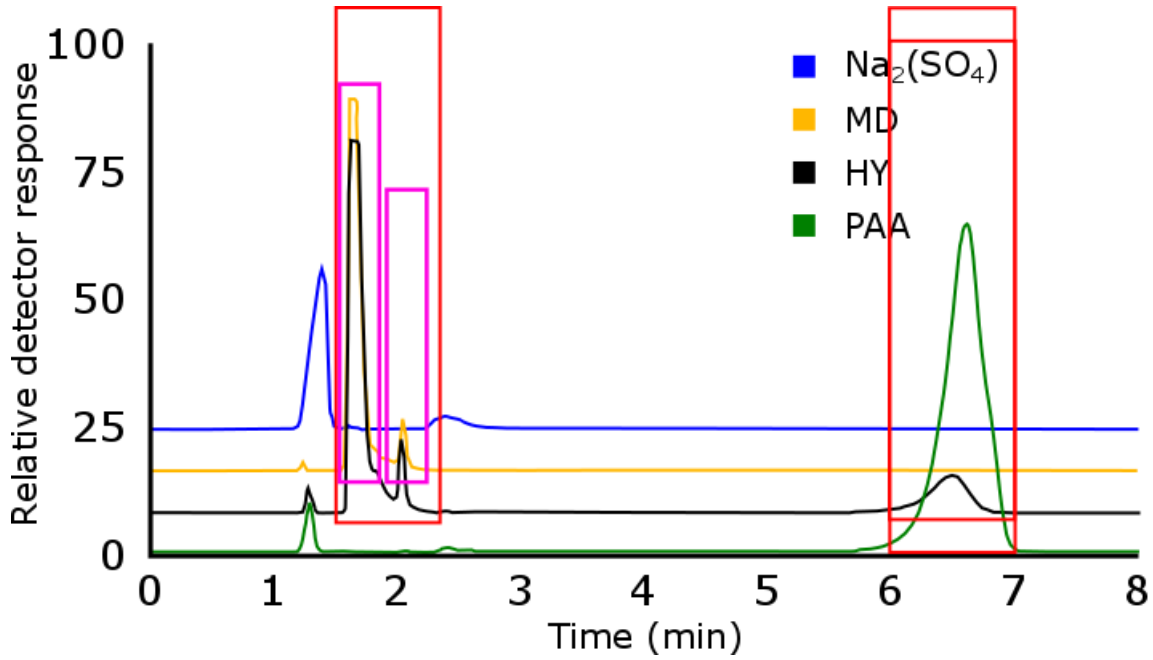

Figure A1. Fractionation windows of polar-embedded RPLC separations. Red boxes denote the collected peaks from MD, HY and PAA. These fractions were then processed and analysed by FT-IR (S2.1) and mass spectrometry (MS) (Figures A3–A5). Magenta boxes of MD sample denote the two fractions analysed exclusively by MS (S2.2, Figure A3). Column: platinum EPS C18 (250 × 4.6 mm i.d., 5-µm particles); mobile phase A: 100% H_2O (0.1% FA); mobile phase B: 100% ACN (0.1% FA) Flow

rate: 2 mL/min; gradient: 3–30% B, (3 to 7 min); detection: ELSD. Chromatograms are shifted in the y-direction for clarity. Detection: ELSD.

S2 Identification

S2.1 FT-IR

Peaks of interest from the reversed-phase separation were collected as fractions post-separation and analysed using FT-IR spectroscopy recorded from 500 to 4000 cm^{-1}. These were MD:1.5–2.5 min (yellow oil, dried on ATR prior to measurement), PAA: 6–7 min (white crystalline powder deposited on ATR) and HY:6–7 min (yellow oil, dried on ATR prior to measurement). An IR blank (grey trace Figure A2) was recorded from the mobile phase sampled from the eluent bottle. The FT-IR spectra are compared in Figure A2. Four absorption bands were considered critical: 1) O-H stretch at 3000–3500 cm^{-1} related to MD (also present in HY), 2) C=O double-bond stretch at 1691 cm^{-1} associated with carboxylic-acid functionality of PAA (present in HY), 3) C-O stretch at 1165 cm^{-1} related to carboxylic acid functionality of PAA (present in HY), and 4) 1005 cm^{-1} ether C-O stretch associated with MD (present in HY). HY shares the C-O stretch at 1000 cm^{-1} with MD. Hybrid and PAA share carboxylic stretches at 1690 and 1164 cm^{-1}, suggesting the presence of PAA in the hybrid.

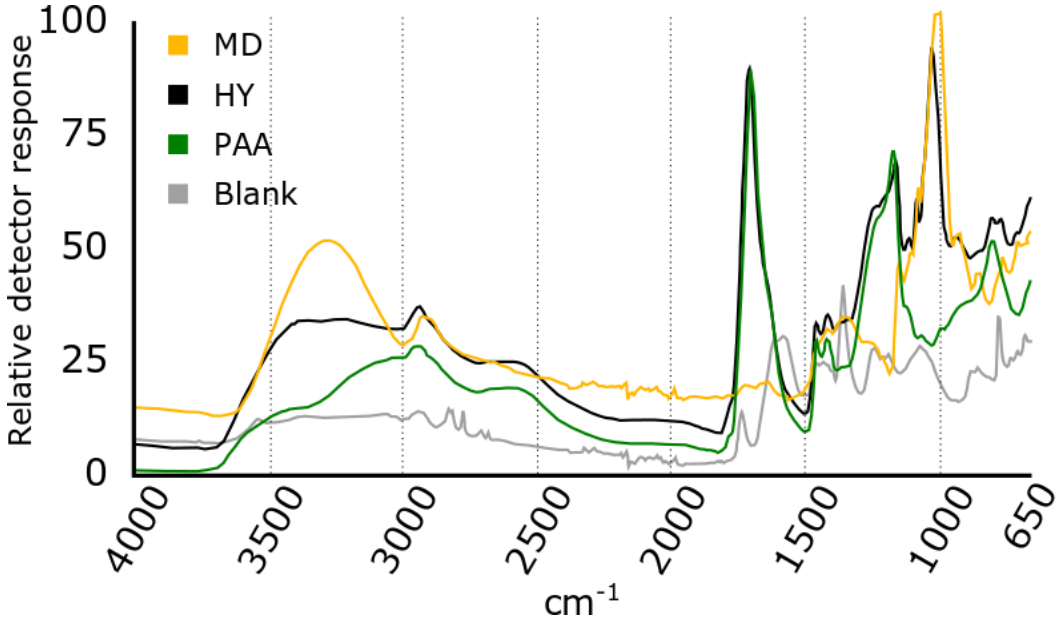

Figure A2. Comparison of the FR-IR spectra of the PAA retained peak (green trace), MD t_0 peak (yellow trace) and HY retained peak (black trace) as well as a blank (grey trace). Spectra were recorded on a Perkin Elmer Frontier spectrometer equipped with a micro ATR accessory.

S2.2 MS

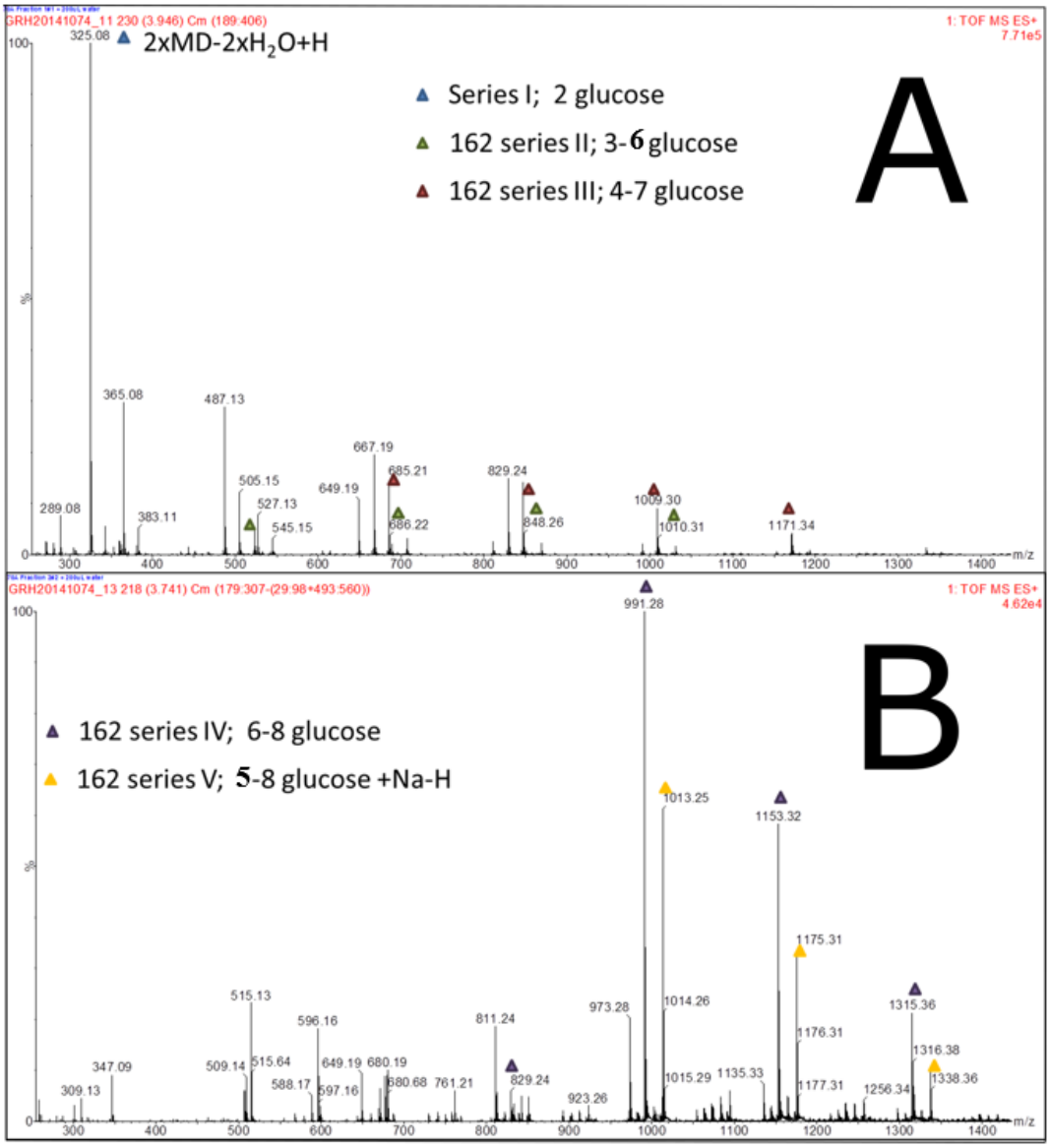

Figure A3. Comparison between MS spectra recorded from MD sample. Spectrum A (upper panel) is the first eluting part of the t_0 peak at 1.8 min in EPS RPLC and spectrum B (lower panel) is a fraction from the slightly retained tail at 2 min. Both spectra show MD oligomers (A 2-5 in 3 series (I, II and III)), B 6-8 in two series (IV, V) and salt adducts. Spectra were obtained on a Waters Xevo (Q-ToF) instrument by direct-infusion analysis of peaks collected from RPLC separation presented in Figure 3 in the manuscript.

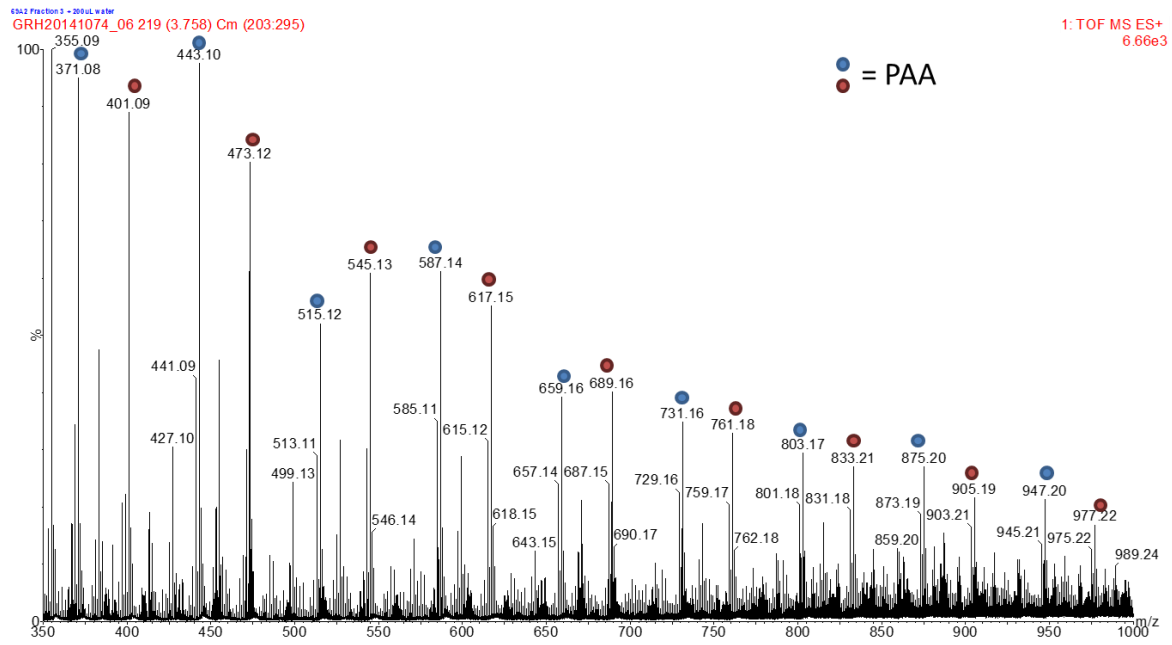

Figure A4. MS spectrum of a retained peak in RPLC separations (6–7 min) from a PAA sample as shown in Figure 3 of the manuscript. The spectrum shows PAA polymer with two types of end groups – saturated and unsaturated. Both PAA polymer series show repeating units with a mass of 72 Da (5–12 monomeric units). No masses corresponding to maltodextrin coupled to (poly)acrylic acid (PAA-MD) were present. Spectra were recorded by direct infusion from collected peaks from RPLC separation presented in Figure 3 in the manuscript using a Waters Xevo(Q-ToF) mass spectrometer.

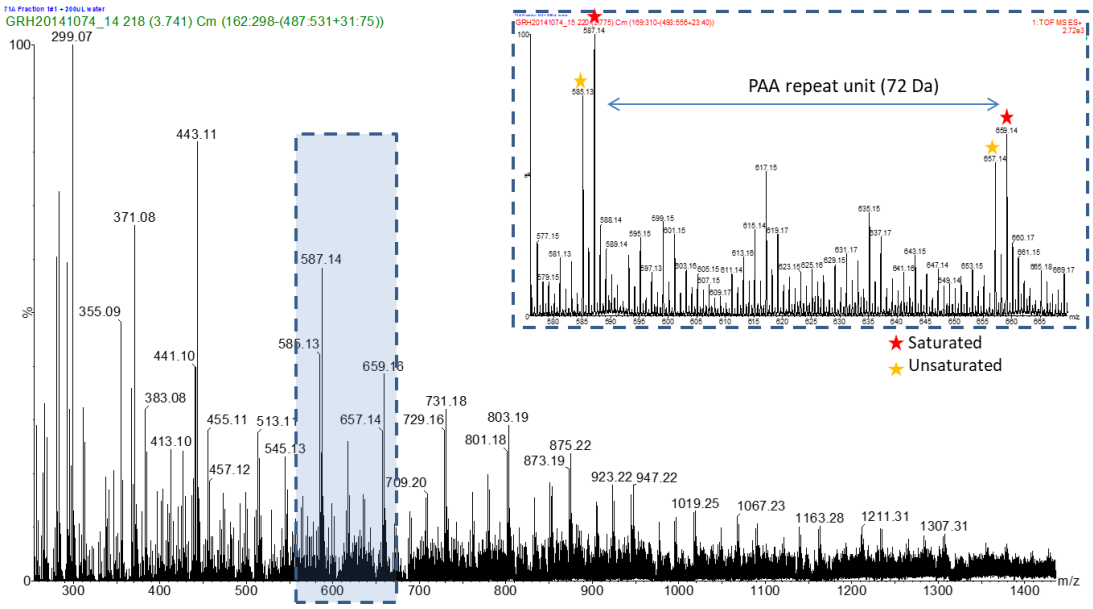

Figure A5. MS spectrum recorded from a retained peak in RPLC separations (6-7 min) from an HY sample as shown in Figure 3 of main text. The spectrum shows PAA polymer with two types of saturation. Both PAA polymer series show repeating units of 72 (5-18 monomeric units). No PAA-MD masses could be found. Spectra were recorded by direct infusion from collected peaks from RPLC separation presented in Figure 3 in the manuscript using a Waters Xevo(Q-ToF) mass spectrometer.

Table A1. List of detected and assigned mass fragments to MD and PAA series.

	MD (Figure A3)				PAA (Figure A4)			
Series	**Label**	**Measured Difference**	**Theoretical Difference**	**MD Units ($C_6H_{10}O_5$)**	**Label**	**Measured Difference**	**Theoretical Difference**	**PAA Units ($C_3H_4O_2$)**
	(m/z)	**(m/z)**	**(m/z)**		**(m/z)**	**(m/z)**	**(m/z)**	
I	**Blue**				**Blue**			
	325.08			2	947.2			12
II	**Green**				875.2	72	72.06	11
	1010.31			6	803.17	72.03	72.06	10
	848.26	162.05	162.14	5	731.16	72.01	72.06	9
	686.22	162.04	162.14	4	659.16	72	72.06	8
	524.1	162.12	162.14	3	587.14	72.02	72.06	7
III	**Red**				515.12	72.02	72.06	6
	1171.34			7	443.1	72.02	72.06	5
	1009.31	162.03	162.14	6	**Red**			
	847.25	162.06	162.14	5	977.22			13
	685.21	162.04	162.14	4	905.19	72.03	72.06	12
IV	**Purple**				833.21	71.98	72.06	11
	1315.36			8	761.18	72.03	72.06	10
	1153.32	162.04	162.14	7	689.16	72.02	72.06	9
	991.28	162.04	162.14	6	617.15	72.01	72.06	8
	829.24	162.04	162.14	5	545.13	72.02	72.06	7
V	**Yellow**				473.12	72.01	72.06	6
	1338.36			8	401.09	72.03	72.06	5
	1175.31	163.05	162.14	7				
	1013.25	162.06	162.14	6				

S3 Chromatography

S3.1 Porous graphitic carbon (PGC) as a stationary phase

The initial testing of PGC stationary phase showed promising results in the separation of polyacrylic acid (PAA) from MD. When injected as aqueous solution, PAA elutes in a single peak at t_0 (1.2 min), while MD oligomers are retained (Figure A6). The MD sample, as well as the hybrid polymer (HY), show a large peak at 1.5-2 min corresponding to glucose (i.e., the monomer of the MD), the dimer elutes at 3.3-4 min and oligomers at 7.5-10 min (Figure A6). The peak in PAA at t_0 was thought to be either unretained additives and/or unretained PAA. Later, the t_0 peak was shown to be caused by unretained additives while the PAA moieties and perhaps the larger MD oligomers did elute from the column under conditions used in this experiment. These analytes eluted at high pH (8.0) and a high ACN concentration (50%) while regenerating the column. This indicates that more information may be obtained on the elution of the HY sample and the pH-dependent selectivity changes. However, it was not possible to develop a reliable separation using these conditions, due to repeatability and recovery problems.

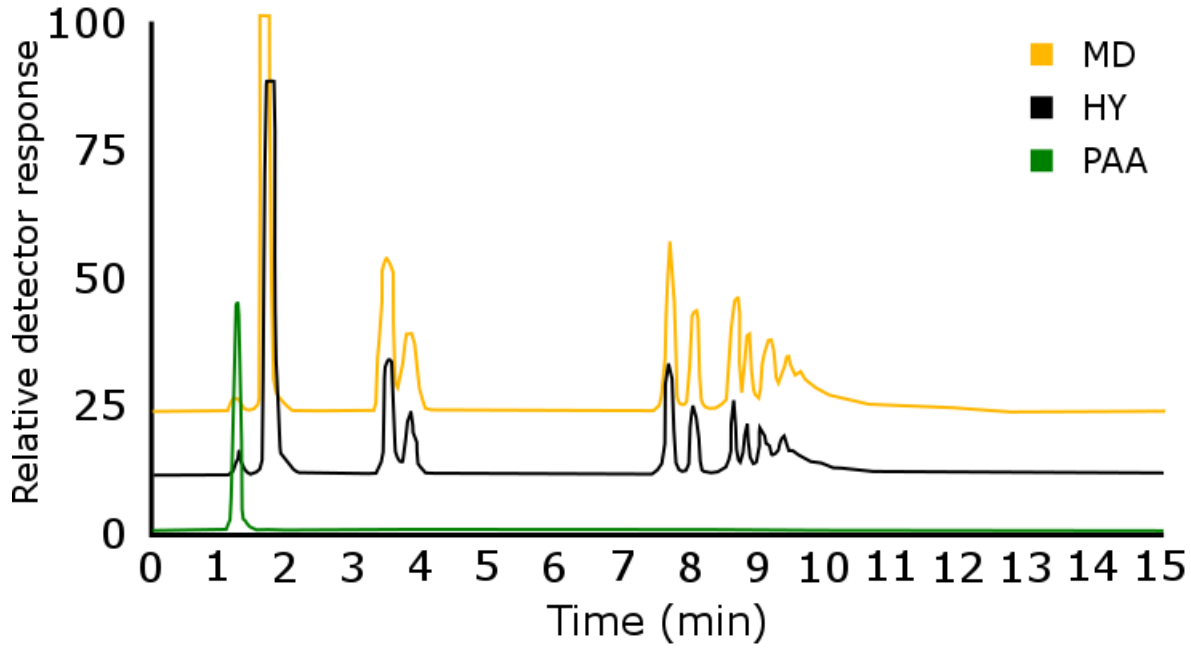

Figure A6. HPLC chromatogram of HY, MD and PAA obtained on a PGC column (Thermo Fisher Hypercarb, 100 × 2.1 mm i.d., 3.0-µm particles). Mobile phase A: 100% H_2O (0.1% FA); Mobile phase B: 100% ACN (0.1% FA) Flow rate: 0.3 mL/min; Gradient 5–35% B, (2 to 10 min); Detection: ELSD. Chromatograms are shifted in the y-direction for clarity. Detection ELSD. Analyte loading: 0.05 mg in 20-µL mobile phase, A.

S3.2 PGC×RPLC

From the one-dimensional separation (Figure A6), the PAA was shown to either elute unretained at t_0 or be absorbed on the column. However, the fact that the MD can be eluted with a maximum of 35% ACN indicated that PGC could be more compatible with ^{2}D RPLC than HILIC. In an effort to provide further insight in the composition of the hybrid polymer sample, PGC was coupled with RPLC as a second dimension. In Figure A7, the LC×LC chromatograms of PAA, MD and HY samples are shown. In Figure A7A, PAA can be seen to give rise to only a single peak around t_0, which is likely caused by salts. If PAA were to elute at t_0 in the PGC dimension, it should be retained in the RPLC dimension (Section 3.1.2). This is clearly not the case, and therefore PAA is probably absorbed irreversibly on the PGC column. This suggests that if a hybrid polymer were present it might also not elute under the current gradient conditions. The absence of retained peaks in the RPLC dimension in the LC×LC chromatogram of the HY sample (Figure A7C) suggests that the presumed hybrid polymer has similar retention properties as PAA or MD on the HYPERCARB stationary phase. The MD (Figure

A7B) shows a separation of mono- and oligomers along the PGC dimension, similar to the one-dimensional HILIC separation (Section 3.1.3 of manuscript). As the separation should yield information on the HY sample, the combination of PGC and RPLC is currently considered uninformative.

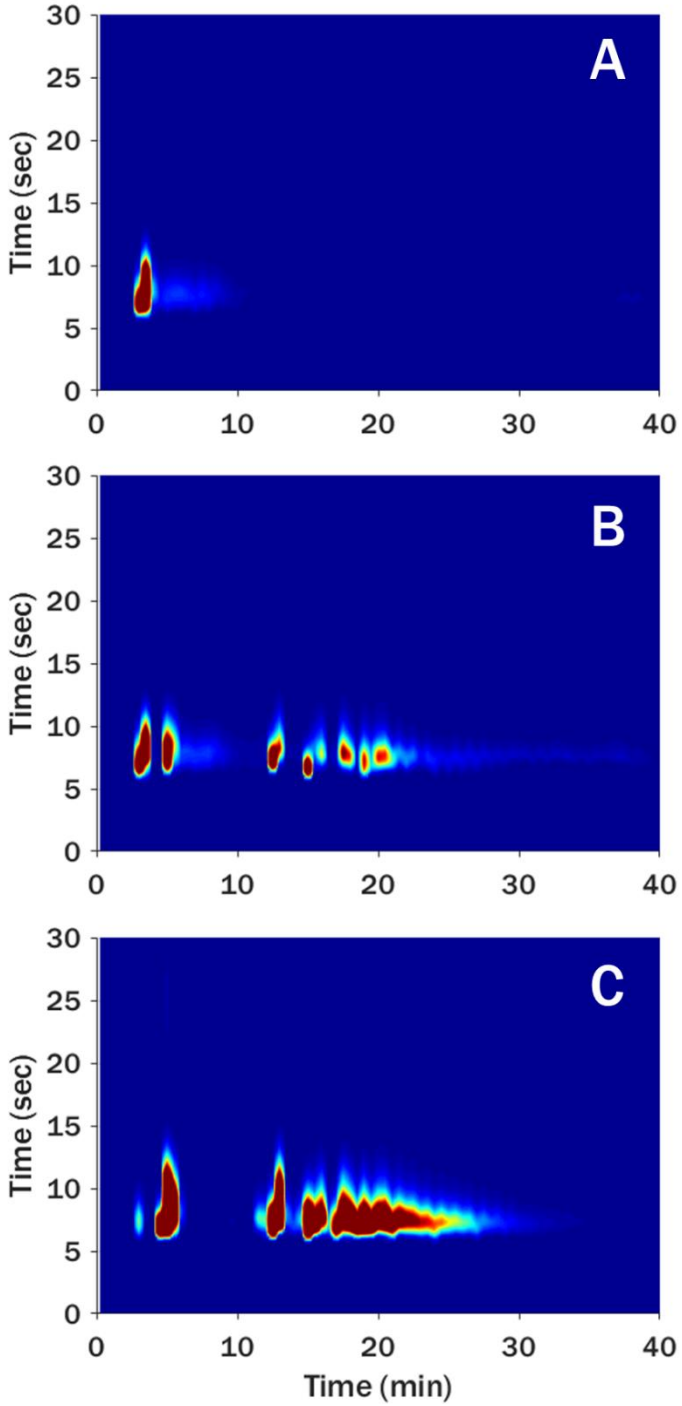

Figure A7. PGC×RPLC analysis (**A**) PAA, (**B**) MD, (**C**) hybrid polymer. A total of 20 µL of 50 mg/mL injection (samples dissolved in water with 0.1% FA). ^{1}D PGC: Thermo Fisher Hypercarb (100 × 2.1, 3 µm), Mobile phase A: 100% H_2O (0.1% FA, 0.25% ACN); mobile phase B: 100% ACN (0.1% FA); flow rate: 0.1 mL/min; gradient: 0-0-30-70-0% B in -0-2-32-37-40 min. Modulation: 50-µL loops; 0.5 min modulation time corresponding to 50 µL modulation volume. ^{2}D RPLC: Titan C18 (30 × 2.1 mm i.d., 1.9-µm particles). Mobile phase A: 100% H_2O (0.1% FA, 0.25% ACN); mobile phase B: 100% ACN (0.1% FA); second-dimension gradient program: 4% to 30% ACN in 0.1 min (starting at 0.1 min), at 1 mL/min. Detection ELSD.

S3.3: Hydrophilic interaction chromatography (HILIC) of MD monomer

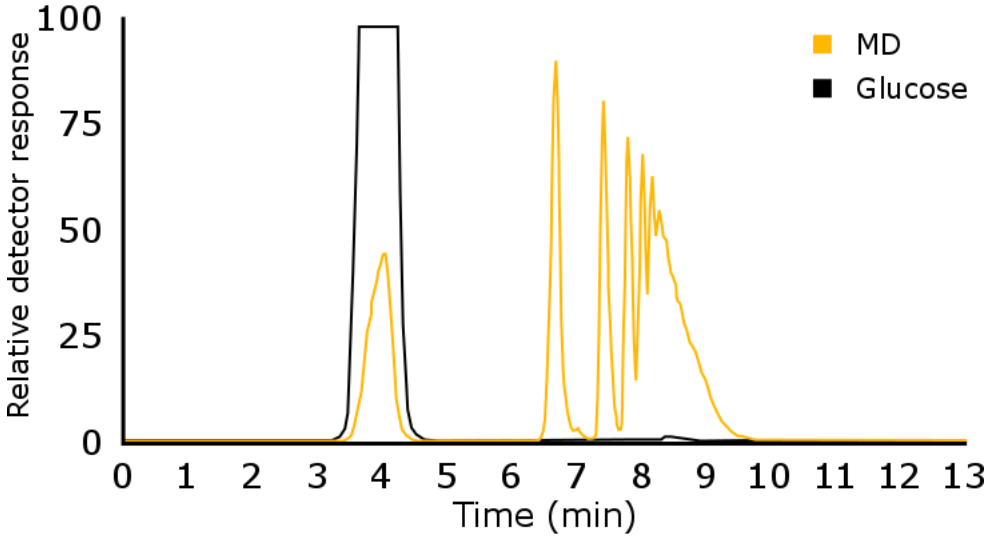

Figure A8. HILIC chromatogram of Maltodextrin (MD) and Glucose standard. 2 μL injections, 8.5 mg/mL solutions. Chromatographic conditions are the same as in the main manuscript (Section 2.2.1.). Glucose has a retention time of 4 min, while t_0 is around 2.2 min. Detection: ELSD.

S3.4 Two-dimensional liquid chromatography

HILIC×RPLC

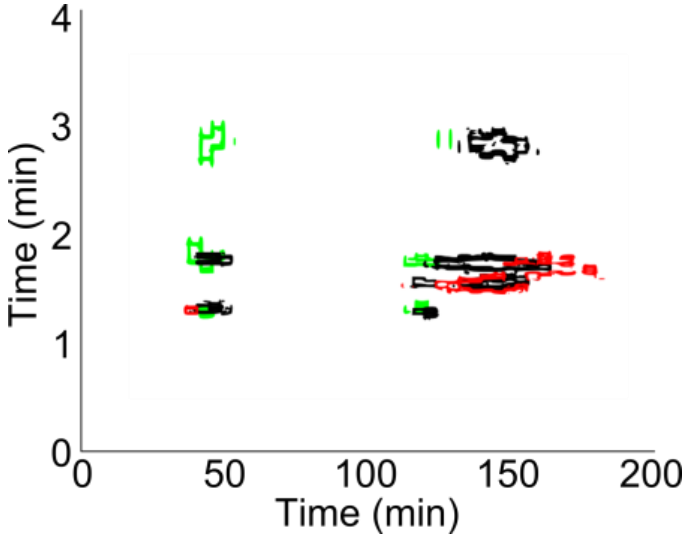

Figure A9. An overlay of the PAA (green), MD (red) and HY (black) HILIC×RPLC chromatograms presented in Figure 8A–C (Section 3.2.2 in the main manuscript, Figure 8). Detection ELSD.

End of appendix.