Supplementary Information

Sequencing of glycosaminoglycans with potential to interrogate sequence-specific interactions

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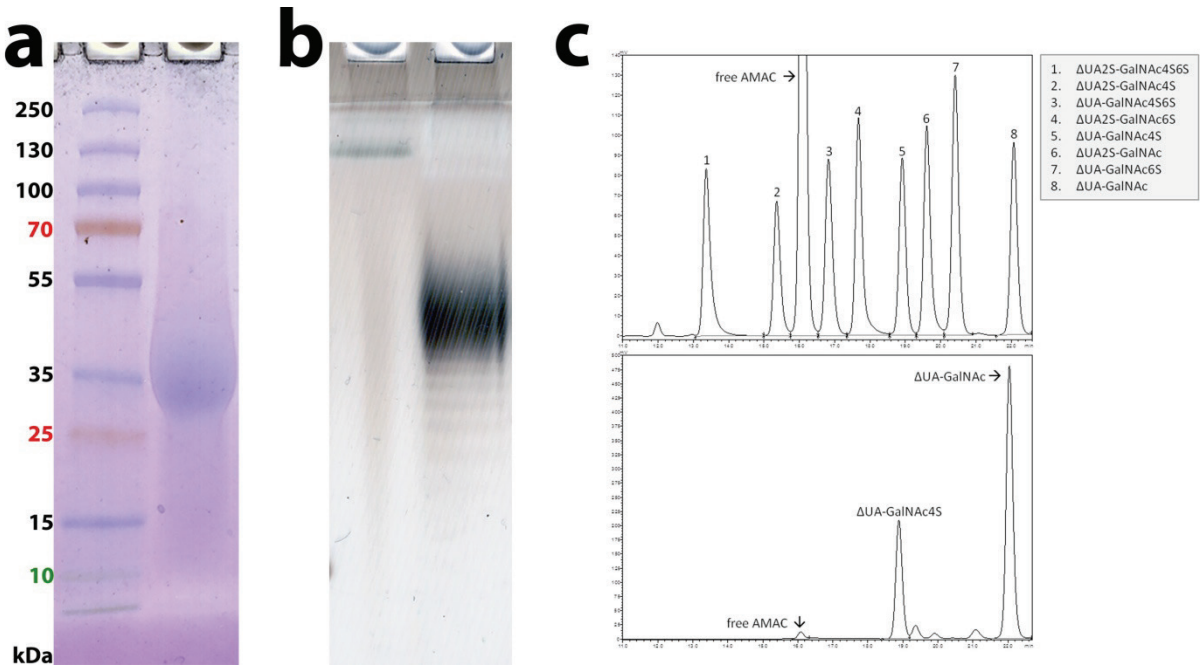
Supplementary Fig. 1.

General characteristics of the bikunin preparation used.

a. SDS-PAGE of bikunin (right lane) showing an apparent molecular weight of about 32 kDa (Coomassie Blue staining). Left lane: molecular weight markers.

b. PAGE of bikunin before (left lane) and after (right lane) alkaline ß-elimination (Alcian Blue/silver staining).

c. RP-HPLC disaccharide analysis of chondroitin sulfate derived from bikunin (chondroitinase ABC digestion, AMAC-labeling, lower panel). Note the presence of ΔUA-GalNAc and ΔUA-GalNAc4S disaccharides. Reference disaccharides are given in the upper panel.



Supplementary Fig. 2.

Gel filtration of end-labeled, fragmented chondroitin sulfate derived from bikunin.

Chondroitin sulfate (~260 µg) derived from bikunin was end-labeled with azidoaniline, treated with chondroitinase AC-II (20 and 60 min combined), and fractionated by FPLC using a Biogel P-6 column. Fractions (2 ml) were collected, lyophilized, subjected to 30% polyacrylamide gel electrophoresis and visualized by incubation with DBCO-IR Dye680RD®. Note that the separation on PAGE is in accordance with the separation on gel filtration column, except for the smallest fragment (arrow) which co-migrates with the fragment obtained by exhaustive digestion with chondroitinase AC-II (lane labeled "60' ACII-ase"), and likely represents the linker region. A selected number of fractions are shown. Note that the fractions A8-B6 and B11-D1 are of two separate gels.