

Assays for determining heparan sulfate and heparin *O*-sulfotransferase activity and specificity

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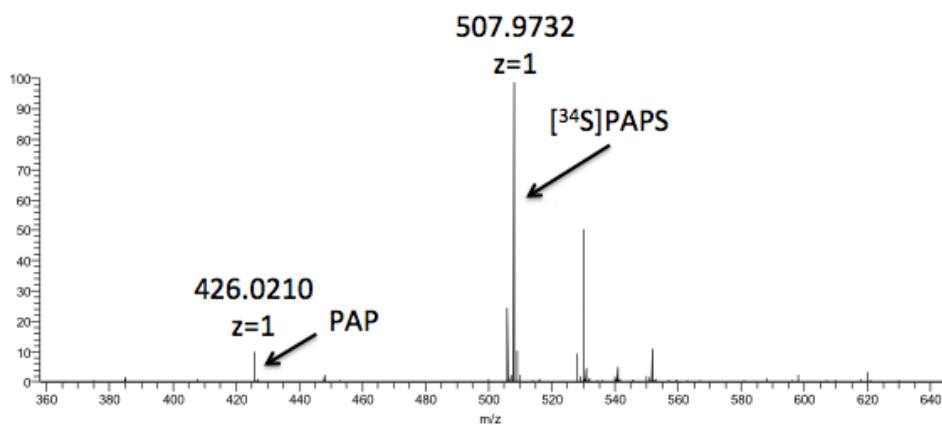


Fig. S1. [³⁴S]PAPS product mass spectrum

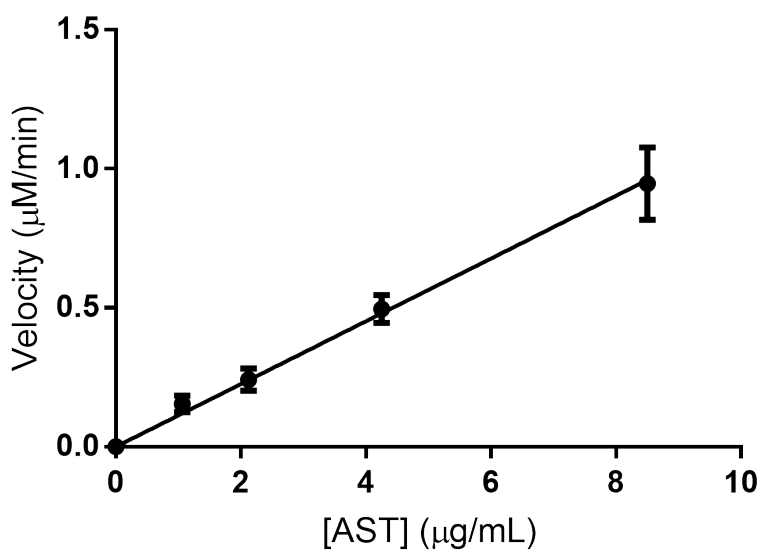


Fig. S2. (A) Linearity of AST-IV initial reaction rate as a function of AST-IV concentration at a [PAP] = 100 μM

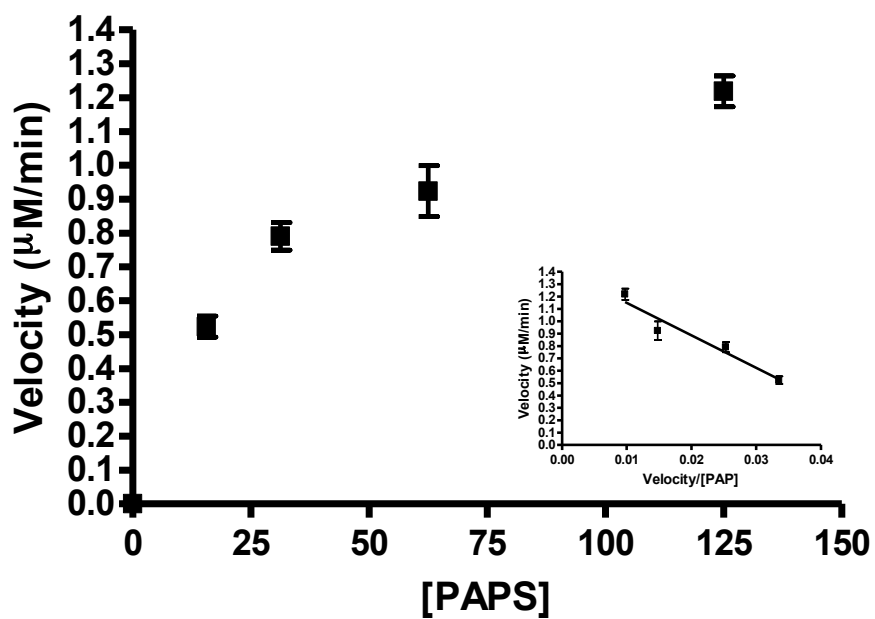


Fig. S2. (B) Michaelis Menten and Eadie-Hofstee (Inset) plots for AST-IV

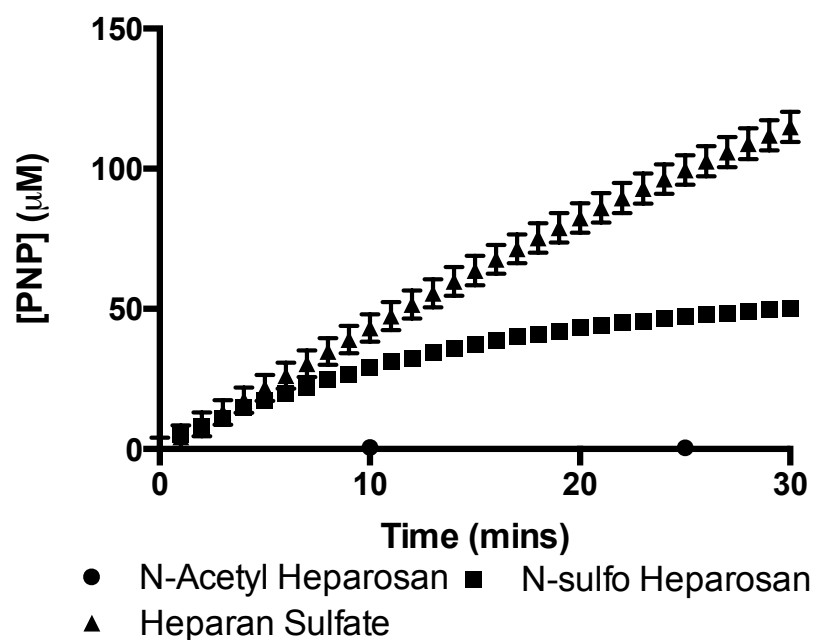


Fig. S3. Comparison of the time course of 6OST-3 on heparan sulfate, *N*-sulfoheparosan, and *N*-acetylheparosan

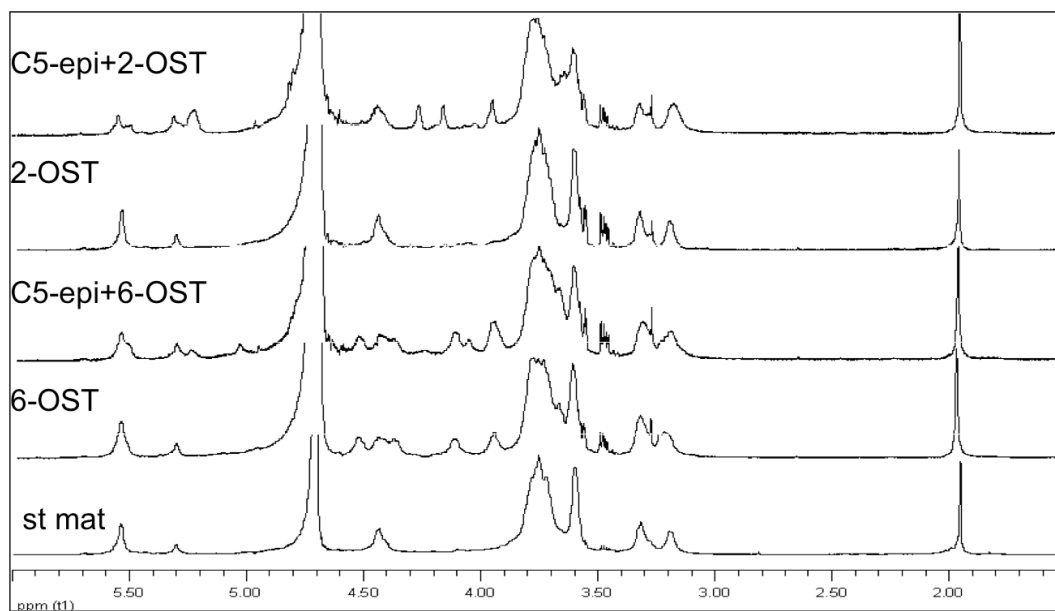


Fig. S4. (A) ^1H -nuclear magnetic resonance spectroscopy of *N*-sulfoheparosan under variable conditions: (1) Addition of C_5 -epi and 2OST; (2) Only 2OST added; (3) Addition of C_5 -epi and 6OST-3; (4) Only 6OST-3 added; (5) *N*-sulfoheparosan starting material

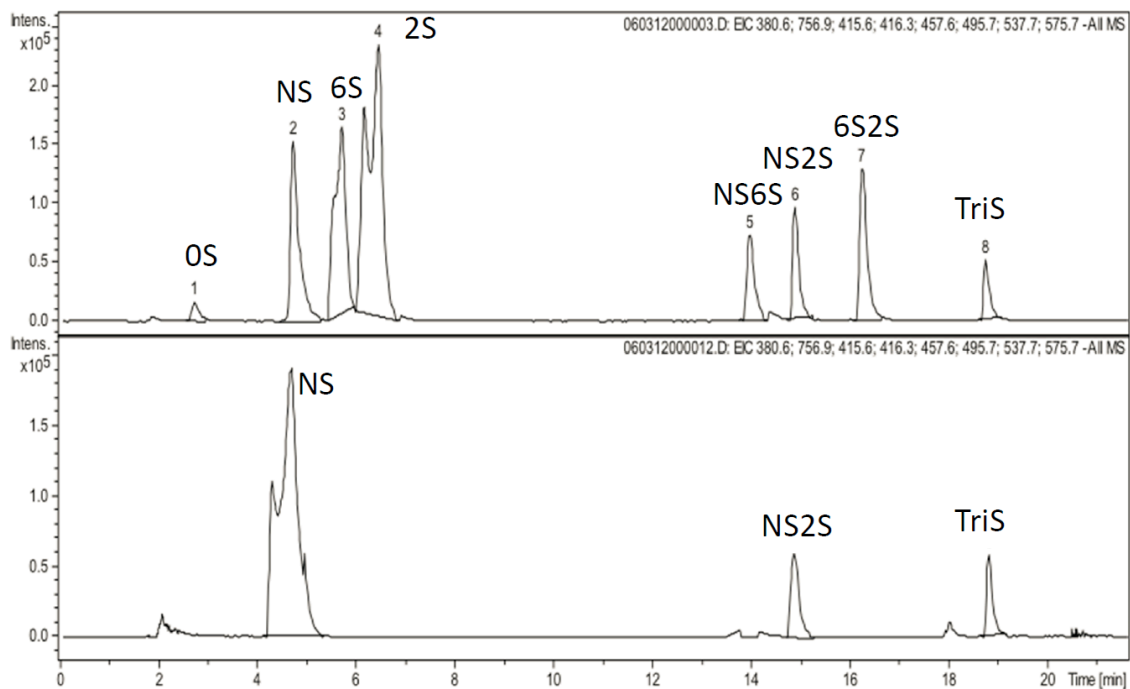


Fig. S4. (B) Liquid Chromatography Total Ion Chromatogram: (Top) Heparin standard; (Bottom) 6OST-3 reaction on partially 2-*O* sulfonated *N*-sulfoheparosan. OS, Δ UA-GlcNAc; NS, Δ UA-GlcNS; 6S, Δ UA-GlcNAc6S; 2S, Δ UA2S-GlcNAc; 2SNS, Δ UA2S-GlcNS; NS6S, Δ UA-GlcNS6S; 2S6S, Δ UA2SGlcNAc6S; and TriS, Δ UA2S-GlcNS6S

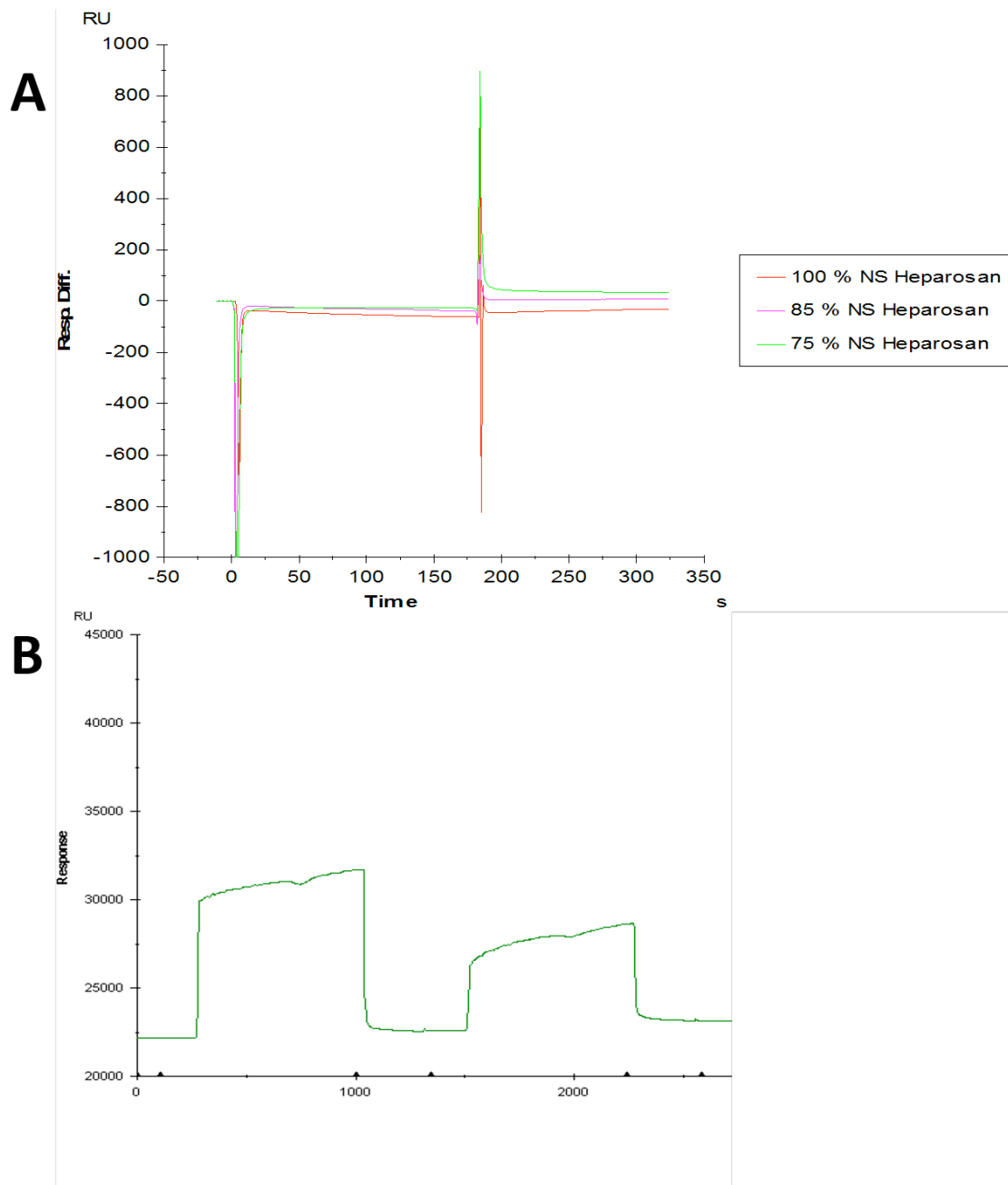


Fig. S5. Surface Plasmon Resonance (SPR) of 6OST-3 and N-sulfoheparosan. A. 6OST-3, in the absence of PAPS, was passed over an SPR chip with N-sulfoheparosan (of varying %NS content) immobilized on the surface. Based on the SPR spectrum, no binding of 6OST-3 was observed in the absence of PAPS. B. 6OST-3, in the presence of PAPS was passed over an SPR chip with N-sulfoheparosan (75 % NS heparosan)

Fig. S6. 6-OST-3 Sequence comparison, (NCBI Reference Sequence: NP_056635.2) The two sequences show an 81% sequence homology with the commercial enzyme possessing an inserted region from aa 28-110

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1 MDERFNKWLL TPVLTLLFVV IMYQYVSPSC TSSCTNFGEQ LRSGEARPPA VPSPARRAQA
61 PLDEWERRPQ LPPPPRGPPE GSRGVAAPED EDEDPGDPEE EEEEEEEEPD PEAPENGLSP
----- 111 PEAPENGLSP
121 RFVPRFNFTL KDLTRFVDFN IKGRDVIVFL HIQKTGGTTF GRHLVKNIRL EQPCSKAGQ
121 RFVPRFNFTL KDLTRFVDFN IKGRDVIVFL HIQKTGGTTF GRHLVKNIRL EQPCSKAGQ
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181 KKCTCHRP GK KETWLF SRFS TGWSCGLHAD WTELTNCVPA IMEKKDCPRN HSHTRNFYI
241 TMLRDPVSR Y LSEWKHVQRG ATWKTS LHMCDGRSPTDEL PTCYPGDDWS GVSLREFMDC
241 TMLRDPVSR Y LSEWKHVQRG ATWKTS LHMCDGRSPTDEL PTCYPGDDWS GVSLREFMDC
301 SYNLANNRQ V RMLADLSLVG CYNLTFMNES ERNTILLQSA KNNLKNMAFF GLTEFQRKTQ
301 SYNLANNRQ V RMLADLSLVG CYNLTFMNES ERNTILLQSA KNNLKNMAFF GLTEFQRKTQ
361 FLFERTFN LK FISPFTQFNI TRASNVDIND GARQHIEELN FLDMQLYEYA KDLFQQRYYH
361 FLFERTFN LK FISPFTQFNI TRASNVDIND GARQHIEELN FLDMQLYEYA KDLFQQRYYH
421 TKQLEHQ RDR QKRREERRLQ REHRAHRWPK EDRA MEGTVT EDYNSQVVRW
421 TKQLEHQ RDR QKRREERRLQ REHRAHRWPK EDRA MEGTVT EDYNSQVVRW

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Key: Encoded transmembrane domain removed to prepare soluble enzyme, Soluble commercial 6-OST-3, Soluble laboratory-prepared 6-OST-3

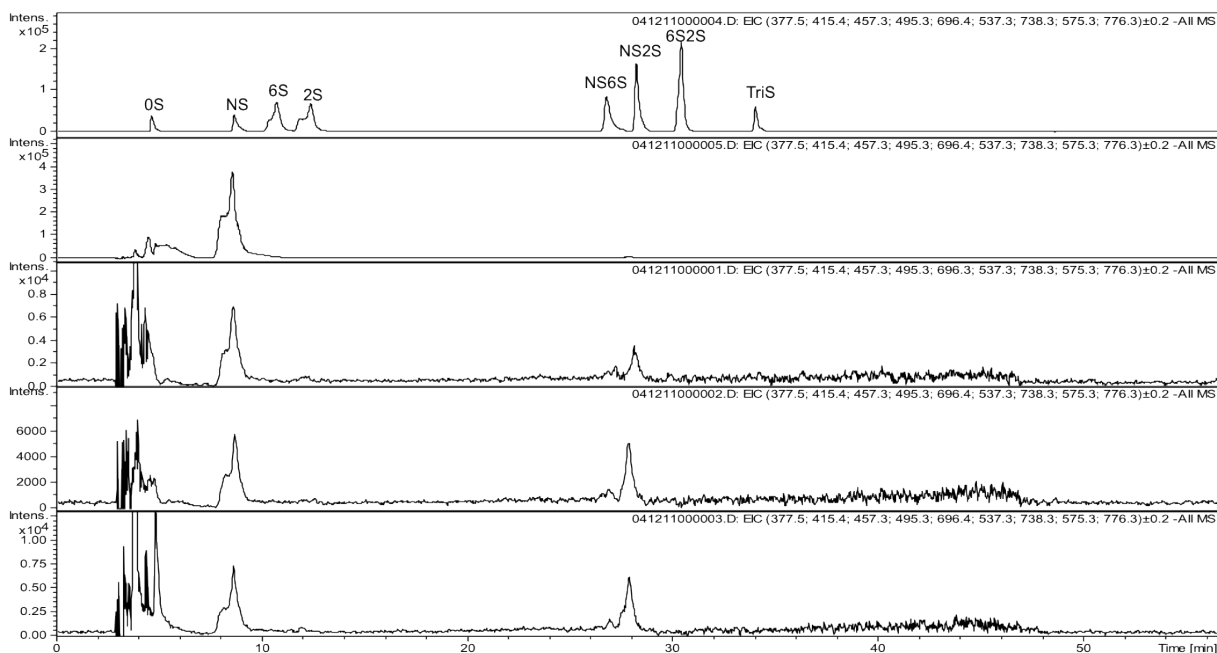


Fig. S7. 2OST reaction on N-sulfoheparosan: (1) Heparin standard; (2) No C₅-epi; (3) 12 h 2OST/C₅-epi reaction; (4) 24 h 2OST/C₅-epi reaction; (5) 48 h 2OST/C₅-epi reaction. See S4 for abbreviations used for disaccharides

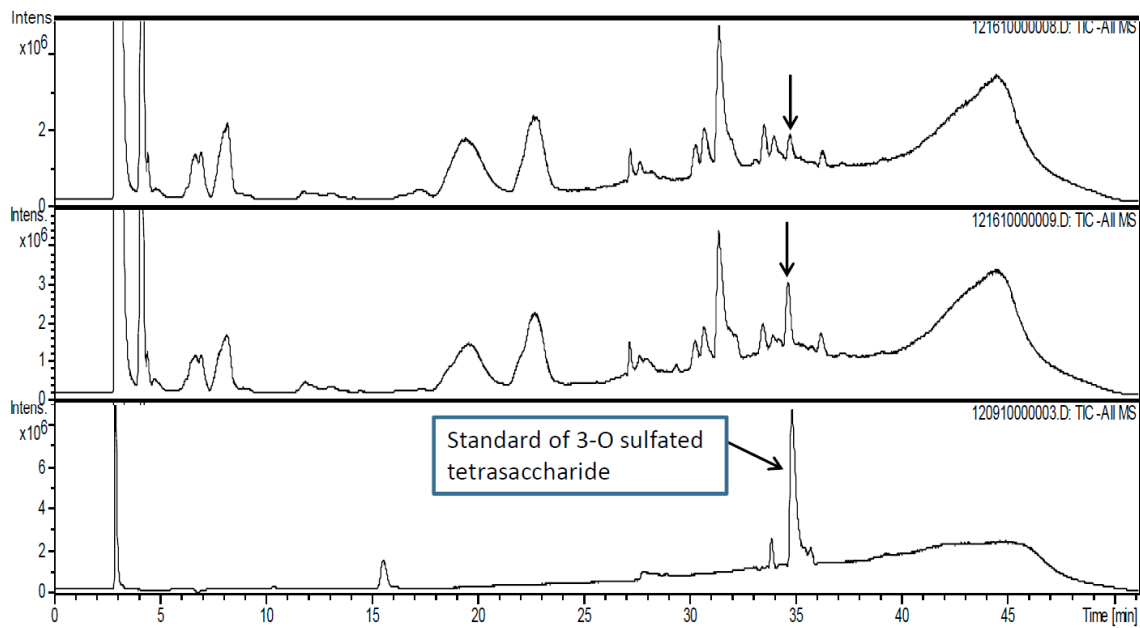


Fig. S8. Liquid Chromatography Total Ion Chromatogram: (Top) Heparan sulfate, (Middle) 3OST-1 reaction on heparan sulfate; (Bottom) Standard for 3-O sulfated tetrasaccharide derived from AT-binding site