**Supplementary Information**

Analysis of substrate sulfation density was carried out utilizing weak anion exchange chromatography (Table S1). Substrates that elute earlier have less charge (fewer sulfate groups) than those that bind more strongly and elute later. Thus, with regard to sulfation density, heparin migrated the slowest on the weak anion exchange column – indicating that it was the most highly sulfated GAG. On the other hand, complete desulfation of heparin using solvolysis drastically lowered its retention time. In order of sulfation density (as determined by the peak retention time at max height), the substrate order is: CDSHep, NA, NS, CDSNS, 2ODS, and heparin (Hep).

| Substrate | Retention Time (min) |
|---|---|
| NA | 20.08 |
| NS | 26.83 |
| CDSNS | 32.61 |
| 2ODS | 51.003 |
| CDSHep | 14.5 |
| Heparin | 51.32 |

**Table S1**. M. Hep retention times as determined by weak anion exchange chromatography.

To analyze the sulfation patterns of the M. Heps, all substrates were digested with heparitinase I, II, and III to yield disaccharide units. These disaccharides were assessed using strong anion exchange chromatography and compared to known disaccharide standards. Heparin predominantly contains ΔIdoA2S-GlcNS6S disaccharides as reported previously.[1, 2] Other abundant disaccharide fragments include ΔUA-GlcNS6S and ΔUA-GlcNS. As expected, 2-O desulfation under alkaline conditions completely converted ΔUA2S-GlcNS6S disaccharides to ΔUA-GlcNS6S disaccharides (2ODS).

Similarly, complete desulfation yielded a polymer containing only ΔUA-GlcNH$_2$ residues (CDSHep). N-resulfation of this substrate yielded a polymer containing ΔUA-GlcNS residues (CDSNS). NS is structurally similar to CDSNS, except that it lacks iduronic acid residues (epimerization). Additionally, CDSHep includes N-amine groups instead of N-Acyl groups and is more positively charged than NA.

To further characterize the molecules, size exclusion chromatography was utilized to probe the molecular weight and polydispersity of the various substrates (Fig. S1). Molecular weight analysis revealed that the NA and NS that we produced are slightly larger polymers than Heparin and CDSHep. Alkaline treatment of HS chains (2ODS) and complete desulfation/N-resulfation (CDSNS) yielded smaller polymers than native heparin. Only minor differences in polydispersity were observed among the library of substrates (inferred from the peak width at half height of size exclusion traces).

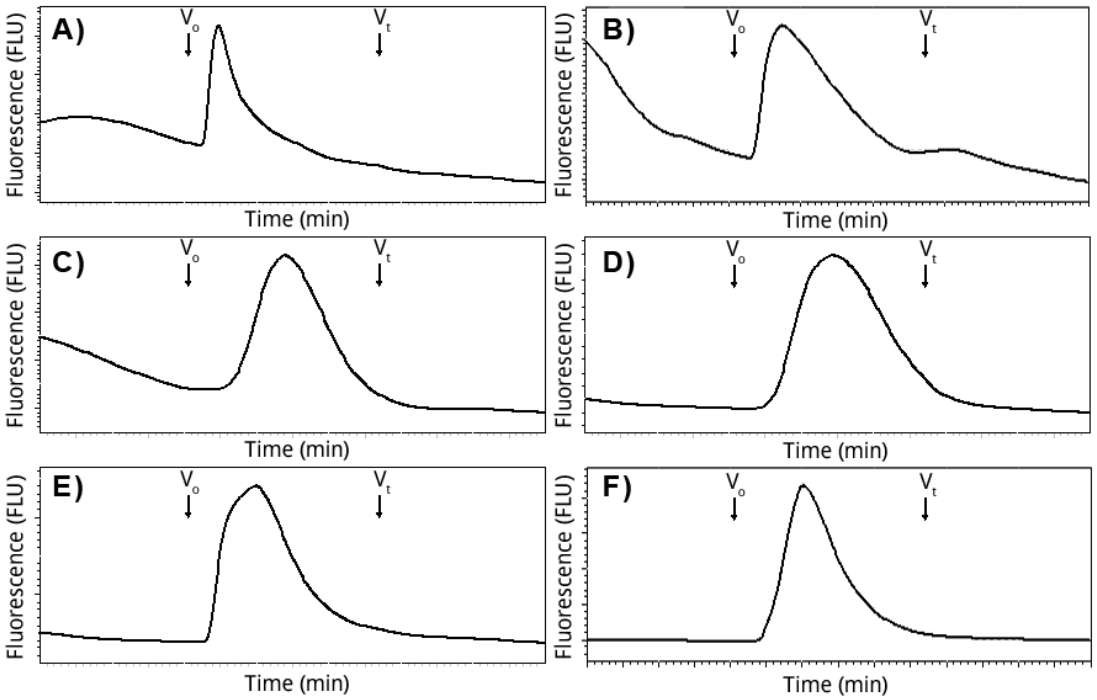


**Figure S1.** Size exclusion chromatography traces of the M. Heps. Substrates that elute earlier are larger in size. Representative traces are: **A)** NA, **B)** NS, **C)** CDSNS, **D)** 2ODS, **E)** CDSHep, **F)** Heparin.

After internalization experiments, we pursued the mechanism of internalization of NS and Heparin into BLMVEC to determine why NS entered cells to a far greater extent than any other substrate (Fig. S2). Previous research has suggested that heparin may enter through receptor mediated internalization using scavenger receptors, but researchers have not determined the internalization mechanisms of NS.[3-5] A series of inhibitors were utilized to determine the mechanism of internalization of NS into BLMVEC. While neither Filipin nor Chlorpromazine had an effect on the uptake of NS, sucrose-, EIPA-, and dynasore- treatment all significantly reduced BLMVEC uptake of NS. It is likely that NS utilizes multiple pathways to enter cells including receptor-mediated endocytosis, dynamin-mediated internalization, and macropinocytosis.

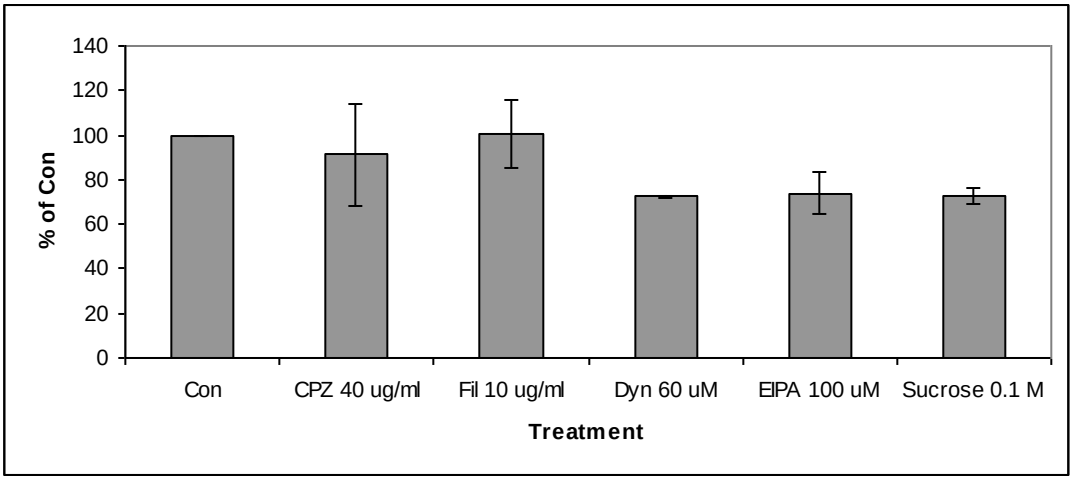


**Figure S2.** NS enters BLMVEC through dynamin, macropinocytosis, and receptor mediated endocytosis. BLMVEC were treated with inhbitors including chlorpromazine (CPZ, a clatherin-mediated endocytosis inhibitor), Filipin (Fil, a caveolin-mediated endocytosis inhibitor), dynasore (Dyn, a dynamin-mediated internalization inhibitor), 5-(N-Ethyl-N-isopropyl) amiloride (EIPA, a macropinocytosis inhibitor), and sucrose (a broad-spectrum receptor-mediated internalization inhibitor). Initially a range of concentrations of each inhibitor were tested. This data represents the maximal inhibition of NS internalization without significant cell death.

To further analyze the mechanism of internalization, a combinatorial treatment with multiple inhibitors was utilized to test if NS uptake could be reduced further (Fig. S3). It was found that combinatorial treatments with multiple inhibitors further reduced uptake of NS and heparin. However, higher dosages of the inhibitors led to significant reduction in cell viability. This reduction in cell viability was expected because Heparan sulfates, analogues of heparin and NS, are rapidly turned over in cells and play integral roles in cell growth and differentiation.[6] Inhibiting multiple pathways that determine NS and

heparin internalization may also interfere with heparan sulfate recycling and thus enhance cell death.

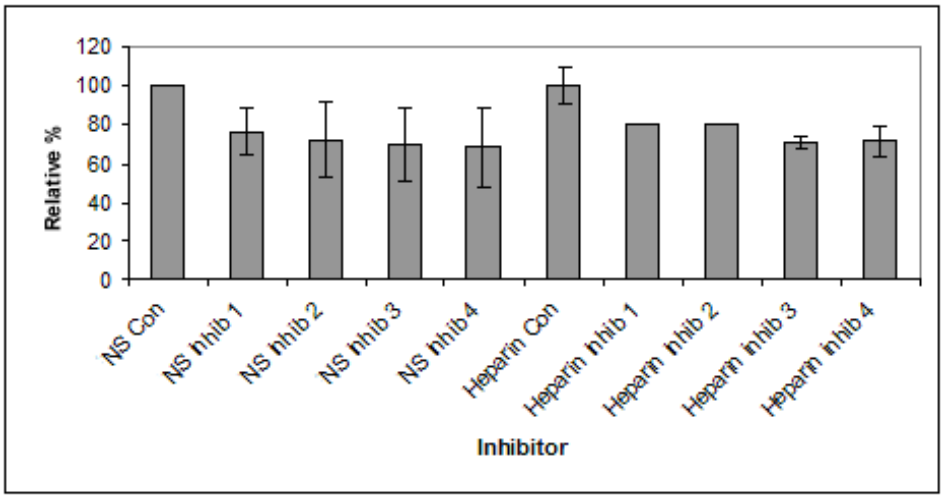


**Figure S3.** Treatment of NS and heparin with a multiple concentrations of a mixture of inhibitors reveals that NS and heparin enter cells by utilizing dynamin, receptor mediated endocytosis, and macropinocytosis. The concentrations of inhibitors tested included: 0.02 M sucrose with 8 uM dynasore and 20 uM EIPA (inhib 1), 0.04 M sucrose with 16 uM dynasore and 40 uM EIPA (inhib 2), 0.06 M sucrose with 24 uM dynasore and 60 uM EIPA (inhib 3), and 0.08 M sucrose with 32 uM dynasore and 80 uM EIPA (inhib 4).

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