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Antithrombin Stabilisation by Sulfated Carbohydrates Correlates with Anticoagulant Activity

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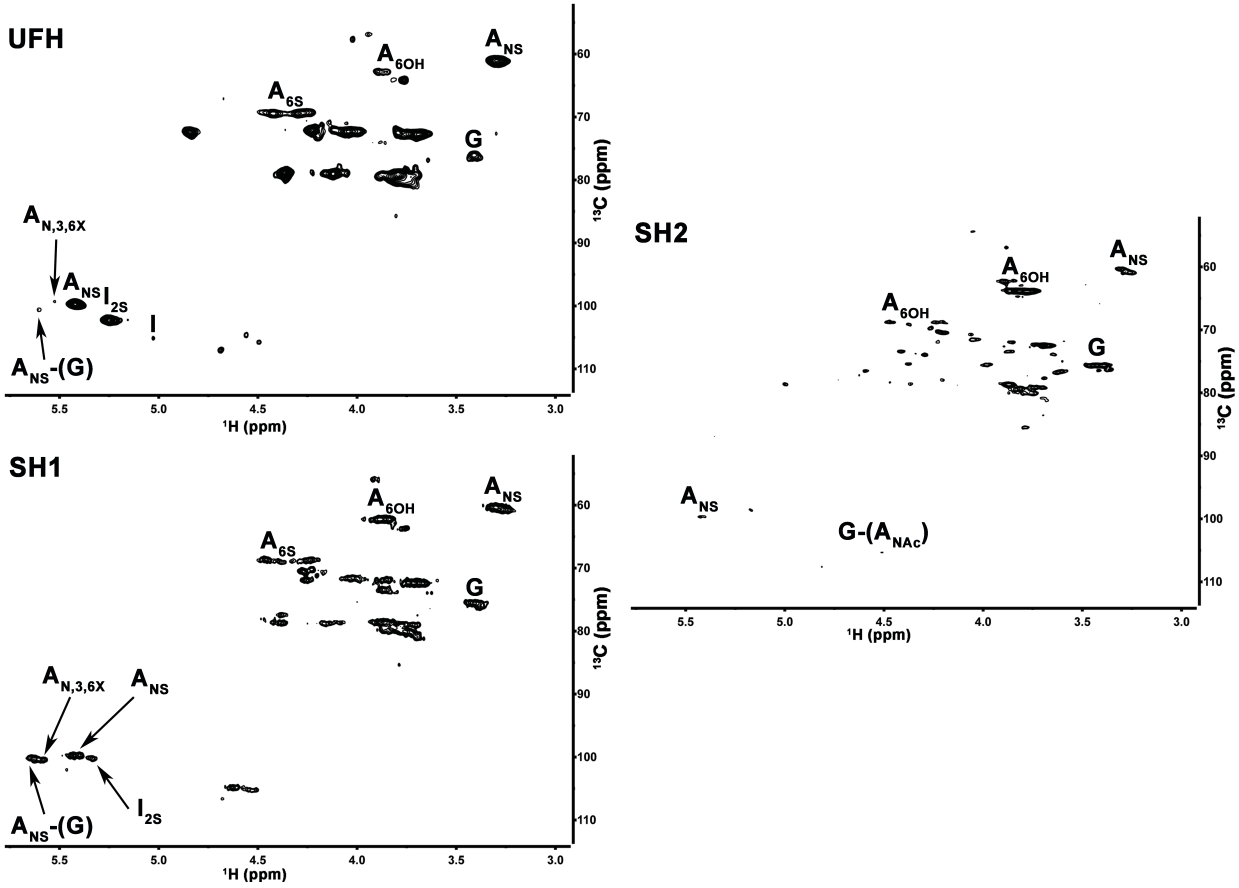
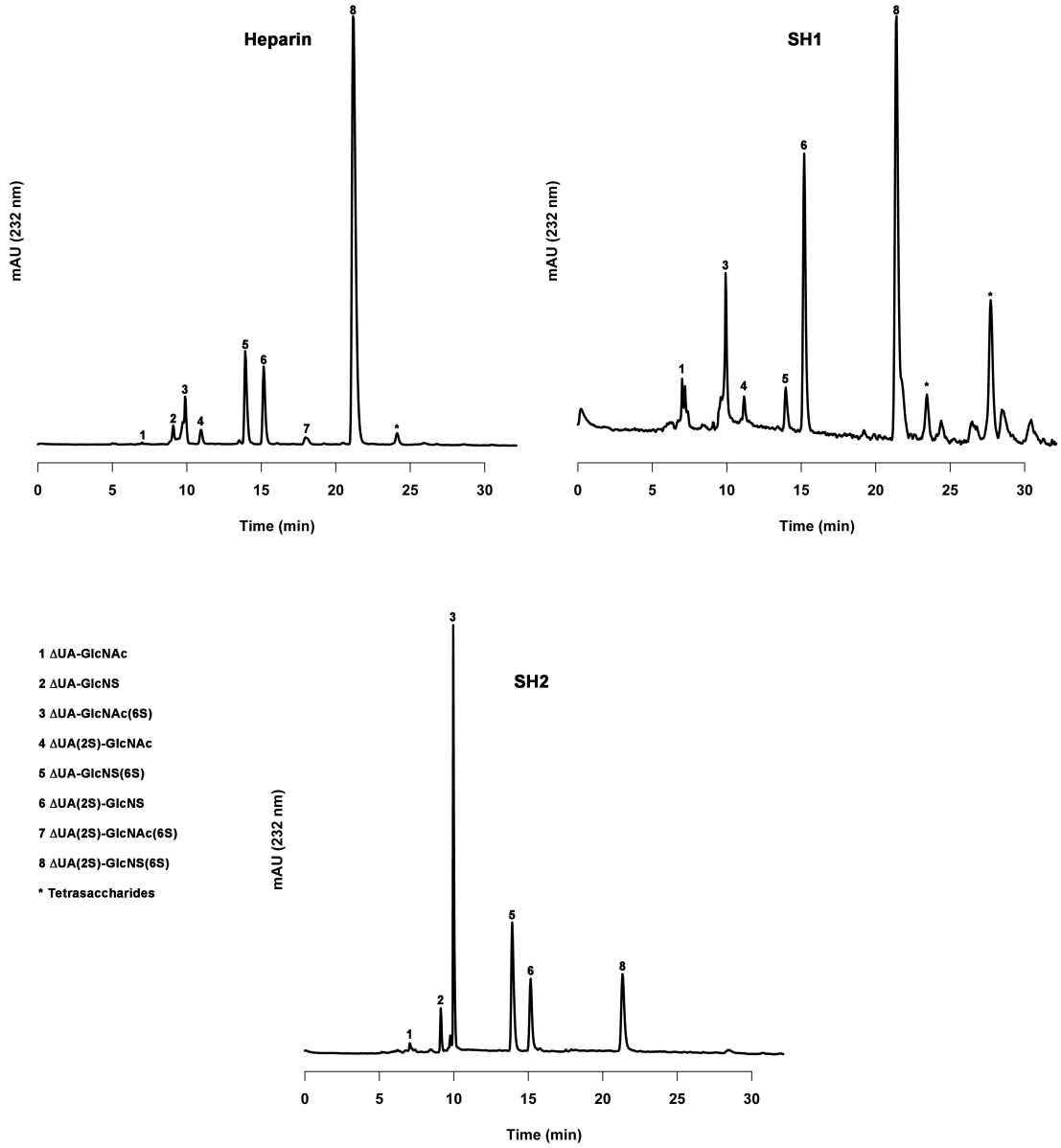
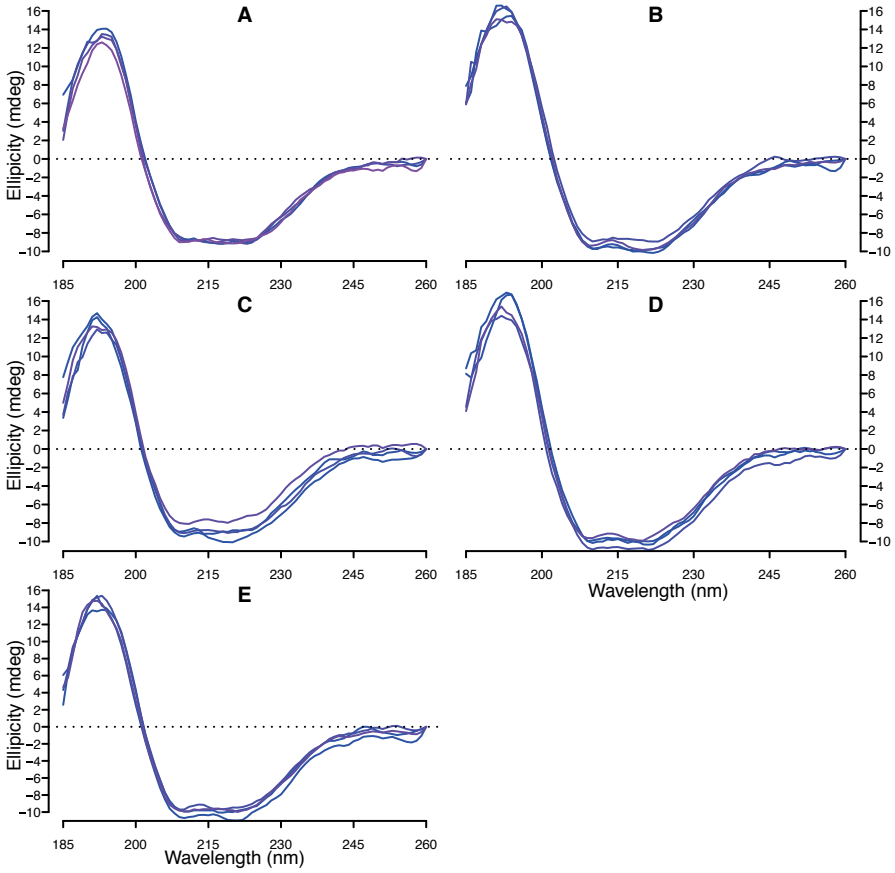


Supplementary figure 1. *^{1}H-^{13}C HSQC spectra (600 MHz) of unfractionated heparin (UFH, upper left), and Shrimp heparinoids-1 (SH-1, upper right) and -2 (SH-2, bottom) from the Pacific White Shrimp, L.vannemei.* Signals from selected structural features are labelled; A_{NS} indicates 2-deoxy-2-sulfamino α-D-glucopyranose; I_{2S}, 2-O-sulfo-α-L-iduronic acid; G, β-D-glucuronic acid; I, α-L-Iduronic acid; A_{3S}, 2-deoxy-3-O-sulfo-2-amino α-D-glucopyranose; A_{NAc}, 2-deoxy-2-acetylamino α-D-glucopyranose. $A_{NS,3S,6X}$ The substitution at position -6 has not been determined.

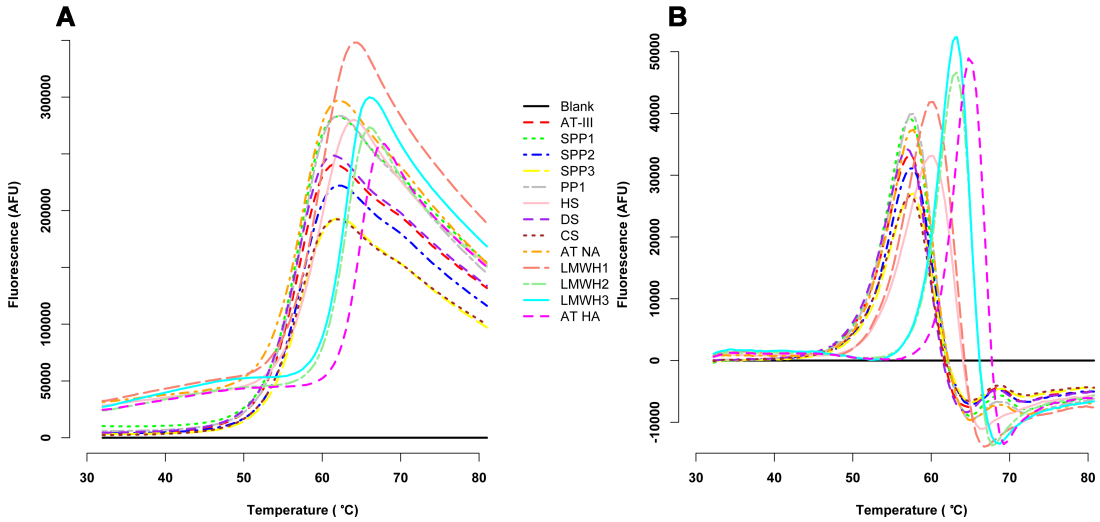


	Disaccharides (%)							
Polysaccharide	**ΔUA-NAc**	**ΔUA-NS**	**ΔUA-NAc(6S)**	**ΔUA(2S)-NAc**	**ΔUA-NS(6S)**	**ΔUA(2S)-NS**	**ΔUA(2S)-NAc(6S)**	**ΔUA(2S)-NS(6S)**
Heparin	0.14	2.07	6.65	1.69	10.33	8.73	1.33	69.07
SH1	9.31	0.00	13.04	1.86	3.45	21.79	0.00	50.56
SH2	1.59	4.83	35.02	0.00	24.75	14.42	0.00	19.39

Supplementary figure 2. *SAX-HPLC disaccharide analysis of unfractionated heparin (heparin, upper left), Shrimp heparinoid-1 (SH1, upper right) and Shrimp heparinoid-2 (SH2, bottom) from the Pacific White Shrimp, L.vannemei, and their disaccharide composition analyses (Table)*. 100 mg of each sample was digested using a mixture of heparin lyases (2.5 mIU each) and analysed on a PhenoSphere™ 5 µm SAX 80 Å LC Column 150 x 4.6 mm.



Supplementary figure 3. *SRCD spectra of AT and AT complexes individual scans used for Figure 1 and 2*. A, AT; B, AT:Pentasaccharide; C, AT:SH1; D, AT:SH2 and E, AT:UFH.



Supplementary figure 4. Detemination of the melting temperature of AT alone and AT bound to a range of polysaccharides via the Fluorescence shift assay. **A.** Raw data and **B.** First derivative. These melting temperatures are compared to the anticoagulant activity of the polysaccharide in supplementary table 2.

Supplementary table 1. *Statistics on secondary structure determination for AT:pentasaccharise and AT:SH2* (Supplementary Figure 4 B and D), illustrating their similar secondary structure content. Numbers in brackets are standard deviations.

	AT:Pentasacchride	AT:SH2
α-helix	85.8 (1.3)	87.3 (1.6)
Antiparallel β-sheet	0.1 (0.1)	0.1 (0.1)
Parallel β-sheet	1.8 (0.2)	1.5 (0.1)
β-turn	8.7 (0.2)	8.8 (0.4)
Unordered	6.3 (0.1)	4.8 (0.6)

Supplementary table 2. List of polysaccharides employed in Figure 2, their melting temperature changes relative to AT and their associated literature references. [a]manufacturers values of activity.

Polysaccharide	Anti-Xa (IU/mg)	T_m (°C) change (from AT)	Reference
SPP 1	≤5	None	1,2
SPP 2	≤5	None	1,2
SPP 3[a]	≤5	None	
PP 1[a]	≤5	None	
HS	~10	+2.6	3
DS	≤5	-1.3	3
CS	≤5	None	
AT NA	≤5	None	4,5,6
LMWH1[a]	100-120	+6.6	
LMWH2[a]	80-120	+8.0	
LMWH3[a]	140-160	+9.3	
AT HA	~400	+12.0	4,5,6

1. Farias EH, Pomin VH, Valente AP, Nader HB, Rocha HA, Mourão PA. A preponderantly 4-sulfated, 3-linked galactan from the green alga Codiumisthmocladum, Glycobiology, 2008, 18, 250-9
2. Rocha HA, Moraes FA, Trindade ES, Franco CR, Torquato RJ, Veiga SS, Valente AP, Mourão PA, Leite EL, Nader HB, Dietrich CP. Structural and hemostatic activities of a sulfated galactofucan from the brown alga Spatoglossum schroederi. An ideal antithrombotic agent? J Biol Chem. 2005, 280, 41278-88.
3. Nader HB, Lopes CC, Rocha HA, Santos EA, Dietrich CP. Heparins and heparinoids: occurrence, structure and mechanism of antithrombotic and hemorrhagic activities, Curr Pharm Des, 2004, 10, 951-66.
4. Nader HB, Takahashi HK, Guimarães JA, Dietrich CP, Bianchini P and Ozima B, Heterogeneity of heparin: characterization of one hundred components with different anticoagulant activities by a combination of electrophoretic and affinity chromatography methods, Int J Biol Macromo, 1981, 3, 356-360.
5. Takahashi HK, Nader HB and Dietrich CP, A method for rapid quantitation and preparation of antithrombin III-high-affinity heparin fractions, Anal Biochem, 1981, 116, 456-461.
6. Takahashi HK, Nader HB, Dietrich CP. High and low anticoagulant activity heparins. Preparation in large scale and degree of complexation with antithrombin III, Arzneimittelforschung. 1985, 35, 1620-3.