SUPPORTING INFORMATION

A Mechanistic Investigation of Methylene Blue and Heparin Interactions and Their Photoacoustic Enhancement

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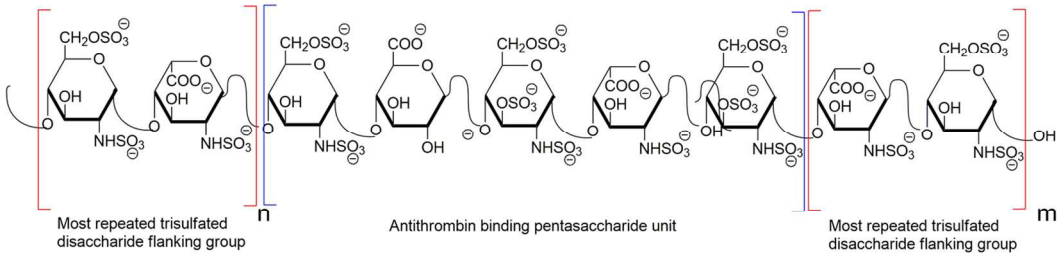
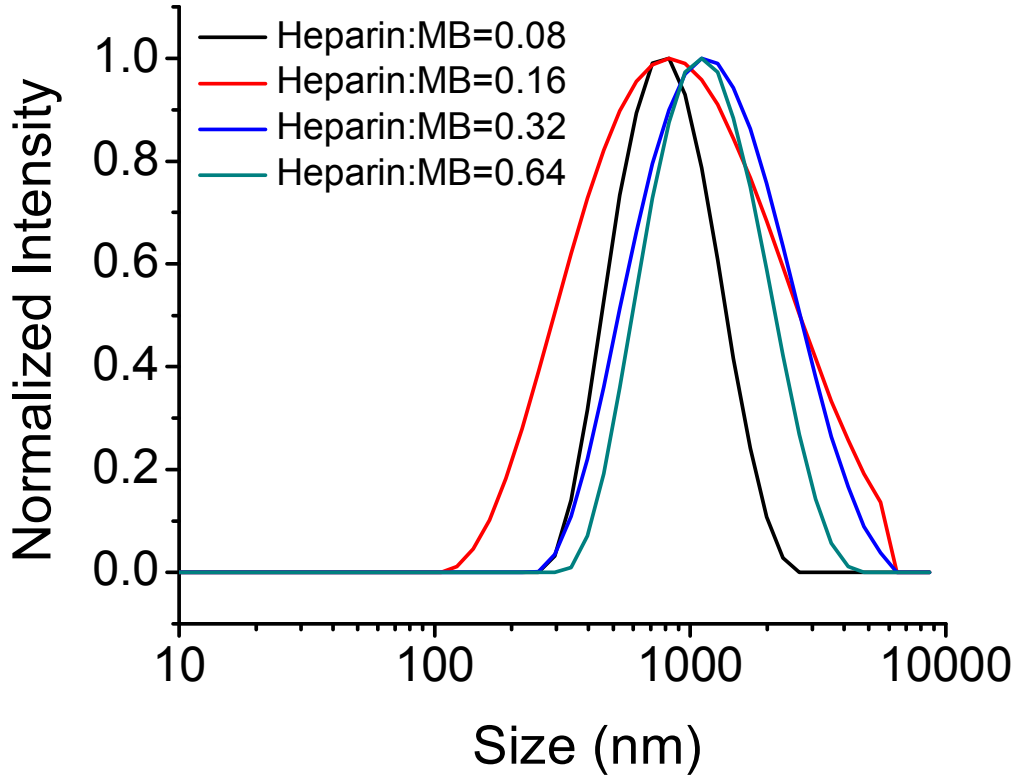
A



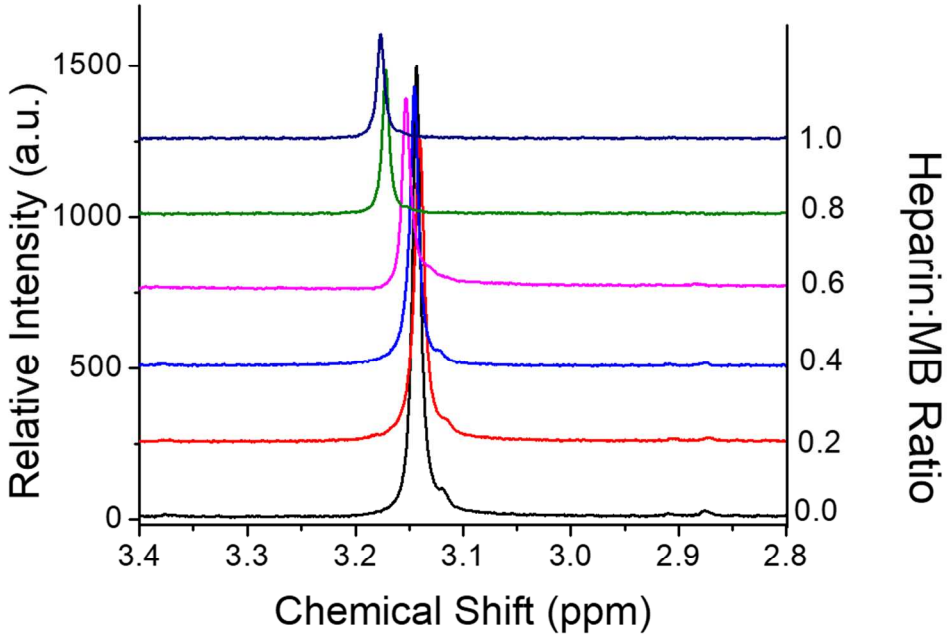
B

Equiv. of heparin	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8
Heparin conc. (mg/mL)	0.09	0.19	0.28	0.38	0.47	0.56	0.66	0.75	0.85

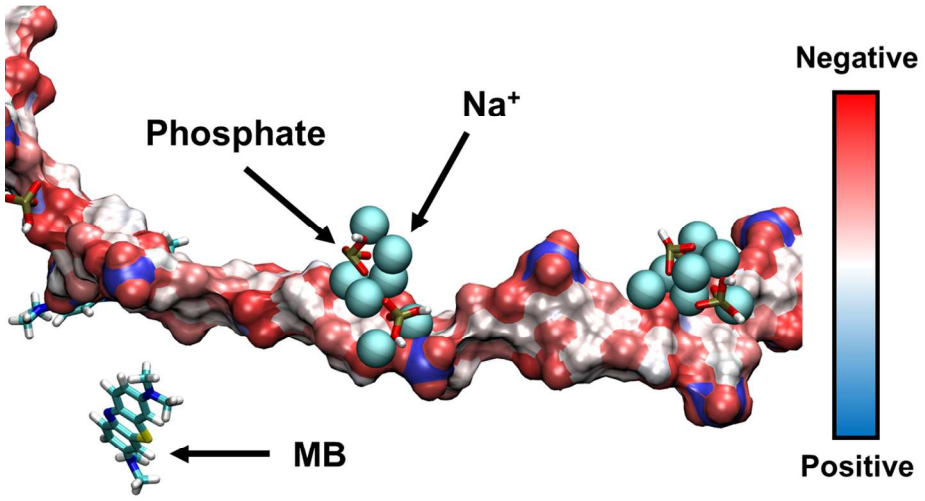
Supplementary Figure S1: Heparin chemical structure and estimated amount of heparin in MB-heparin mixture. Panel A shows the 3 subunits of heparin—an antithrombin binding pentasaccharide site, a flanking disulfated disaccharide, and the dominant repeated bridging trisulfated disaccharide units. Panel B shows the ratio between the estimated number of sulfate groups on heparin and concentration as a function of 0.90 mg/mL MB.



Supplementary Figure S2: Size measurement of the MB-heparin aggregates as a function of heparin concentration. Increasing heparin concentration from 0.03125 to 0.25 mg/mL in 0.2 mM MB increased the MB-heparin aggregate size from 870 nm (0.03125 mg/mL) to 1451 nm (0.125 mg/mL) and then decreased the size to 1279 nm (0.25 mg/mL).



Supplementary Figure S3: NMR spectra of the dimethyl amino proton of the 0.9 mg/mL MB with increasing heparin concentration. The NMR intensity decreased 76% when the heparin:MB ratio increased from 0.0 to 1.0.



Supplementary Figure S4: PBS effect in MB-heparin binding. This figure depicts the interactions between the binding residues of heparin (shown as a surface, red to blue represents charge from positive to negative) and the PBS solvent system (Na^+ ions shown in blue and phosphate ions shown in yellow and red), producing a competition for binding sites between PBS and MB.