Electronic Supplementary Material (ESI) for RSC Advances.

Estimation of Stronger Heparin Binding Locus in Fibronectin Domain III^{14} using Thermodynamic and Molecular Dynamic Study

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Fluorescence quenching studies

Quenching of FN with KI- To find the accessibility of the surface Trp to KI, the fluorescence quenching experiments were carried out using KI in the presence of FN at both the pHs (Fig. S1 (a-b), showing the quenching of fluorophore by KI has limited access to Trp as all the Trp are buried inside the hydrophobic core of the protein. Fig. S1 (c) shows the Stern Volmer quenching constant of FN by KI. K_{SV} for quenching with KI is 0.606 L mol^{-1} at pH 4.0 shown in Table S1.

Quenching of complex (FN and heparin) with KI- Quenching of the complex (FN+ heparin) with KI has similar quenching constant as for free FN at low pH (Fig. S2 (a-b). The Stern Volmer binding plots studies is shown in the Fig. S2 (c).

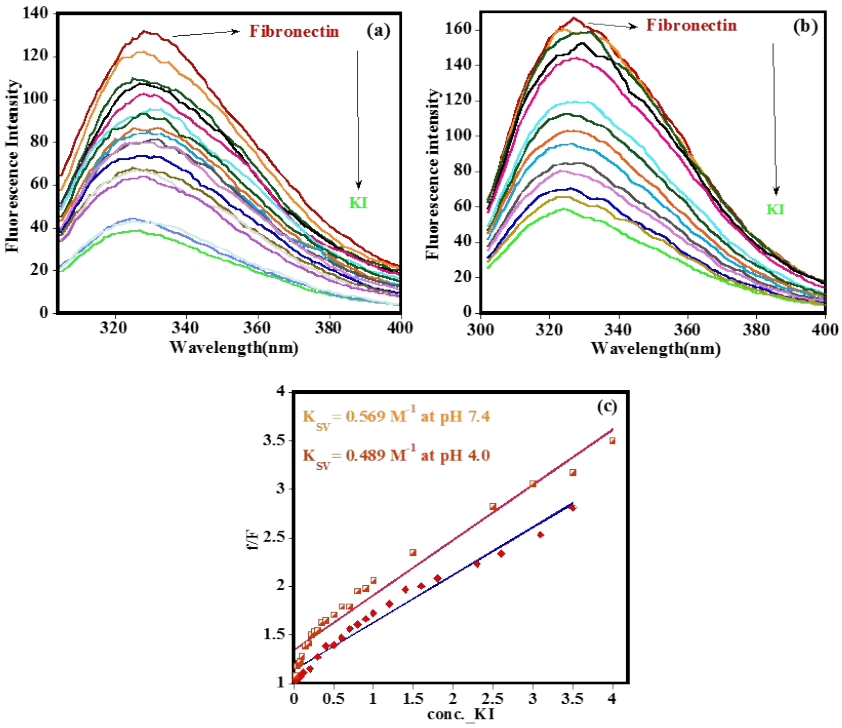

Fig. S1 Fluorescence Emission spectra of FN quenched by KI **(a)** at pH 7.4, FN (1 μM) and KI (20 - 4000 mM) λ_{ems} = 328 nm, salt 150 mM **(b)** at pH 4.0, FN (1 μM) and KI (20 – 2000 mM) λ_{ems} = 326 nm, salt 25 mM, (c) Comparison of quenching of free FN by KI at different pH, shown by the values of Stern Volmer constant.

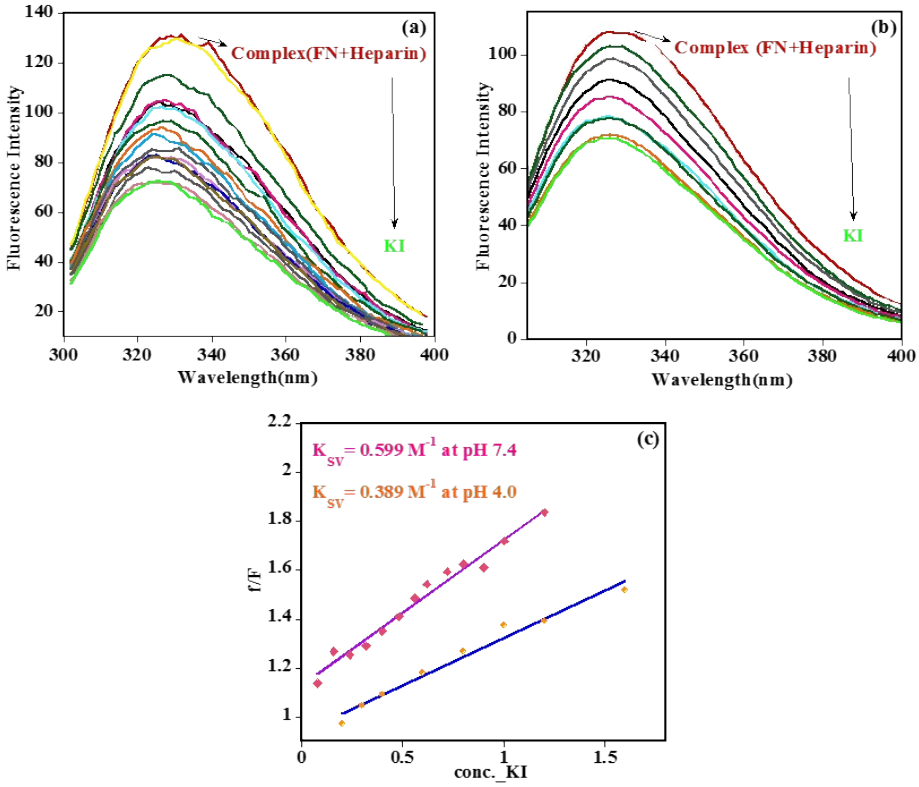

Fig. S2 Fluorescence Emission spectra of complex (FN+heparin) quenched by KI **(a)** at pH 7.4, FN 1 µM + heparin 50 µM and KI (40-1400 mM) λ_{ems} = 328nm, salt 150 mM **(b)** at pH 4.0, FN 1 µM + heparin 5 µM and KI (200-1600 mM) λ_{ems} = 326nm, salt 150 mM, **(c)** Comparison of Stern Volmer quenching constant of FN bound with heparin by KI at different pH.

Table S1 Binding constant and quenching constant values obtained from fluorescence studies at pH 7.4

Componenets used	**pH**	**K_{SV}**
FN + KI	4.0	0.489
	7.4	0.569
FN + Hep + KI	4.0	0.389
	7.4	0.599

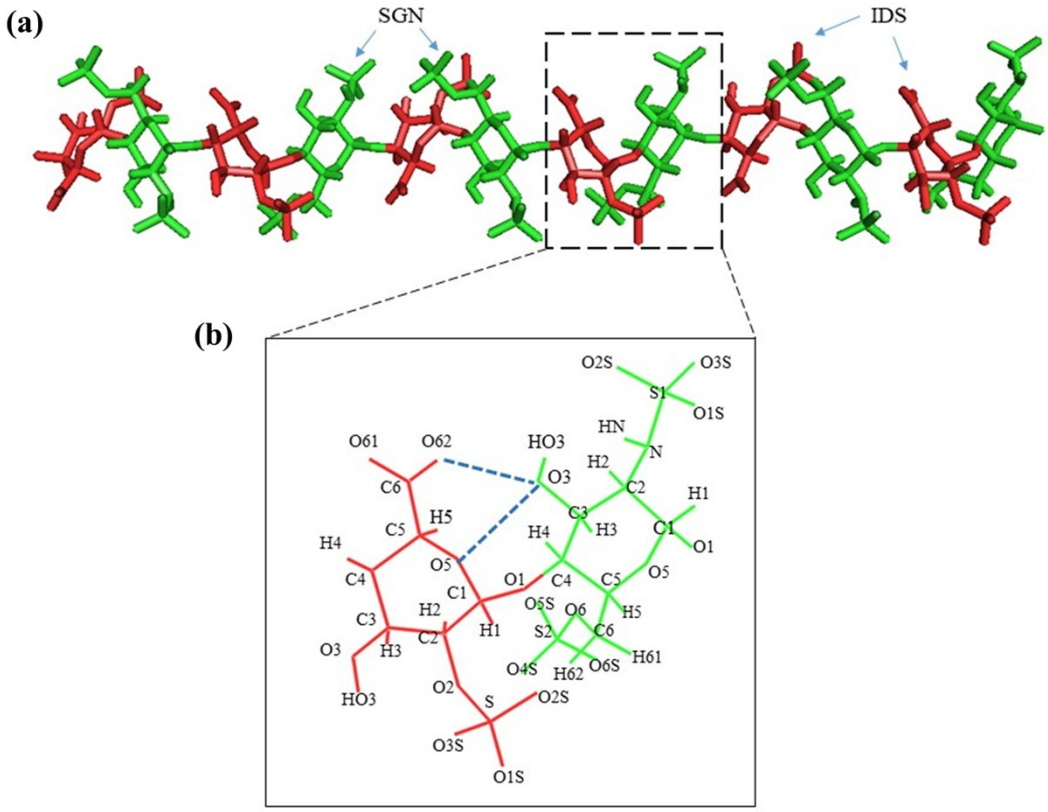

Fig. S3 Structure of heparin (PDB ID 1HPN). **(a)** The dodecasaccharide unit of heparin used as a ligand for molecular docking and MD simulations. It has alternatively arranged six units of N,O6-Disulfo-glucosamine (SGN) and six units of 2-O-sulfo-alpha-L-idopyranuronic acid (IDS) shown in green and red, respectively, **(b)** Interaction between a unit of IDS and SGN in the dodecasaccharide chain.

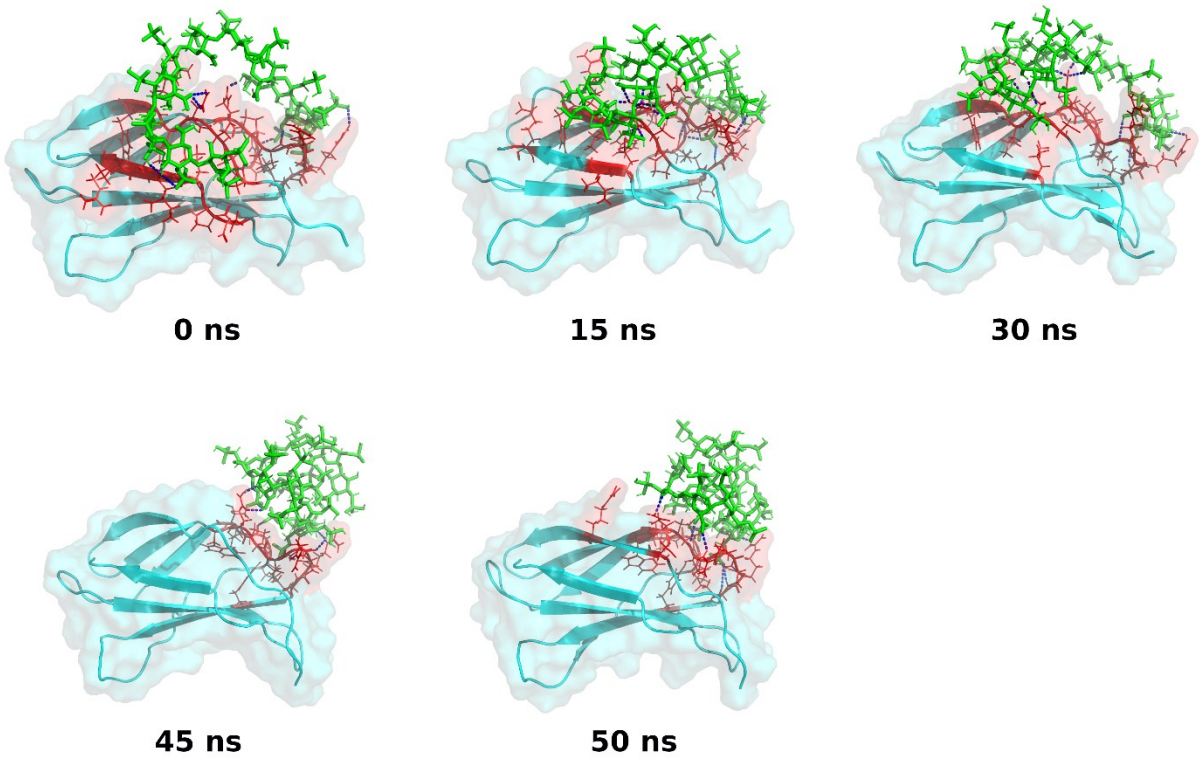

Fig. S4 Snapshots from Molecular dynamics simulation of heparin at FHIP I site of fibronectin at various time step. The region of protein involved in the interaction is highlighted in red color. The hydrogen bonds in each complex are represented by blue color.

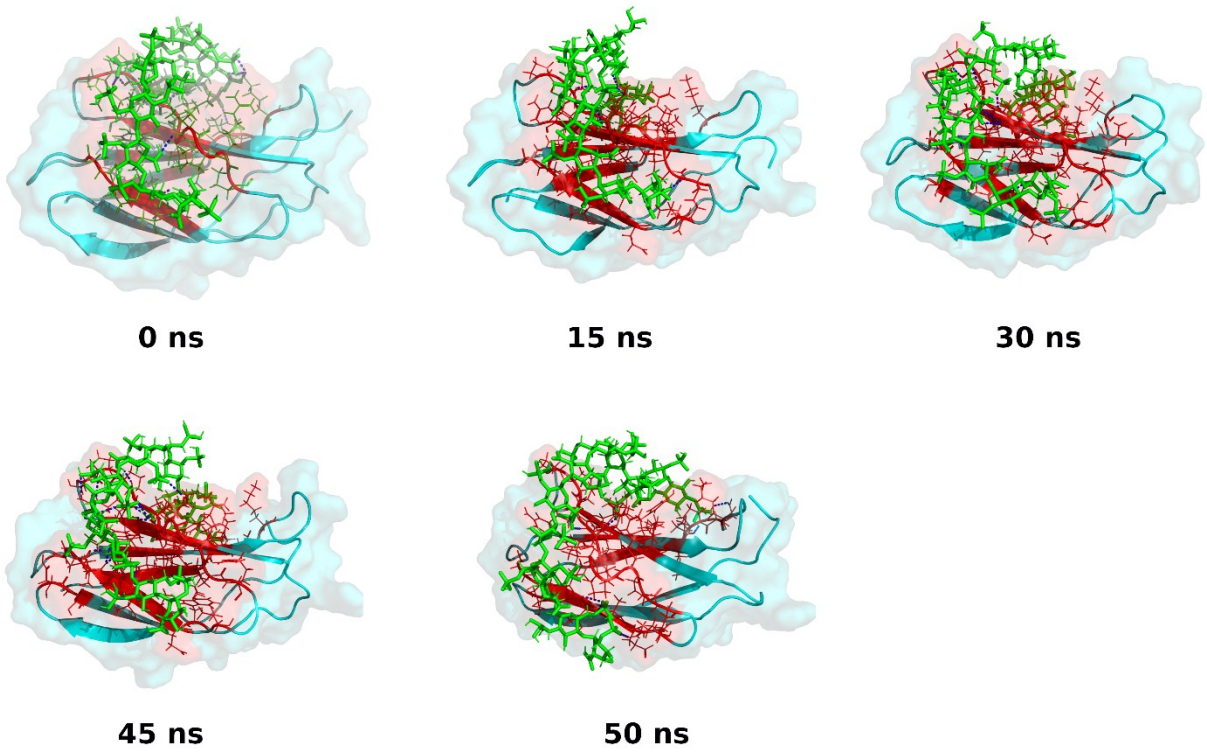

Fig. S5 Snapshots from Molecular dynamics simulation of heparin at FHIP II site of fibronectin at various time step. The region of protein involved in the interaction is highlighted in red color. The hydrogen bonds in each complex are represented by blue color.

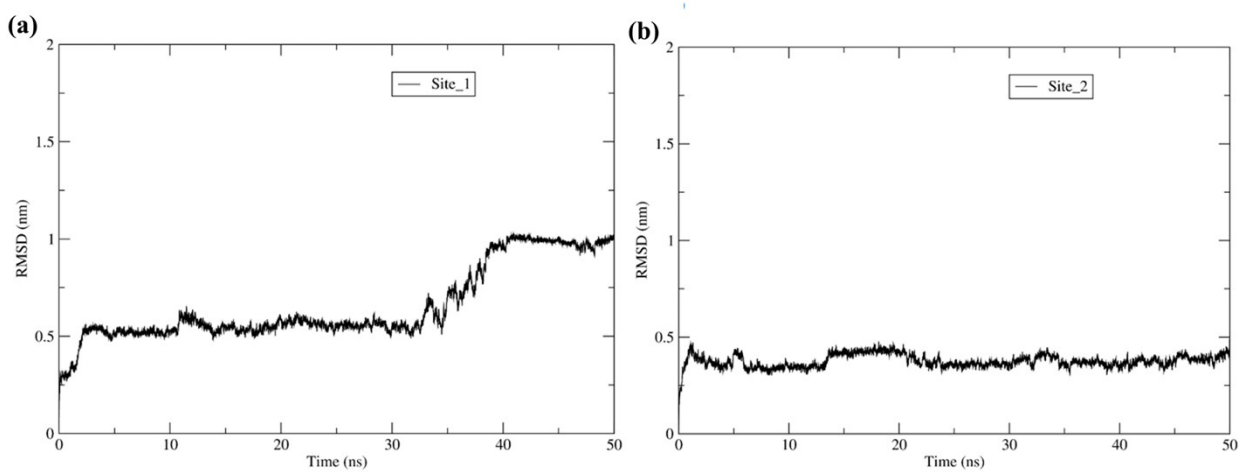

Fig. S6 Root mean square deviation plot (RMSD) plot for ligand (heparin). **(a)** RMSD of ligand when bound at site FHIP I, **(b)** RMSD of ligand when bound at FHIP II.