Biomolecular interaction and the interactome

Glycans serve a critical role in cell-cell communication and function through their interaction with other biomolecules, such as proteins. Glycan-biomolecule interaction has several unique features, including high specificity, low avidity, multi-valency, high on-rates/off-rates of binding and responsiveness to environmental changes.

<u>Microarrays of glycosaminoglycans to probe protein interactome</u>: Glycan microarrays are being constructed containing glycosaminoglycans having different sequences at each spatially addressable location. These glycan chains are either first, purified, structural characterized and then immobilized or are synthesized at site. By probing such glycan microarrays with proteins from a given cell, tissue or organism the interactome can be established.

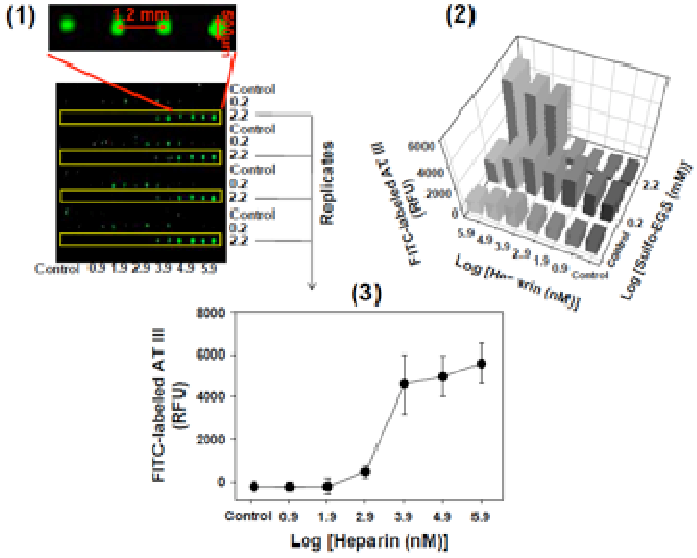

Figure. Fluorescence intensity of bound FITC-labeled antithrombin (AT) as a function of reactant concentrations. (1) A scanning image of the slide where the printed concentrations of heparin varied. Spots containing no FITC-labeled amino heparin were used as control. (2) An average of fluorescent intensity of bound F-labeled AT in the spots at different concentrations of reactants printed. (3) Fluorescent intensity of bound F-labeled AT in the poly L-lysine spots modified with heparin reactant.

<u>Surface plasmon resonance and isothermal titration calorimetry to study protein-carbohydrate interactions</u>: The thermodynamic (ΔH) and kinetic (k_{on}/k_{off}) properties of protein-carbohydrate interactions are important parameters to understand binding. The ΔH, ΔS and stoichiometry of binding can be measured using isothermal titration calorimetry (ITC) while the k_{on}/k_{off} , K_{assoc}, K_{dissoc}, K_{eq} can be conveniently measured using surface plasmon resonance

(SPR). SPR and ITC can also provide information on conformation changes in binding, cooperativity, ionic *vs.* nonionic component of binding, loss of hydrophobic domain exposure to solvent on binding, and loss of counter ions on binding events. Our group expertly uses SPR and ITC to understand the biophysics of interactions between glycans and other molecules.

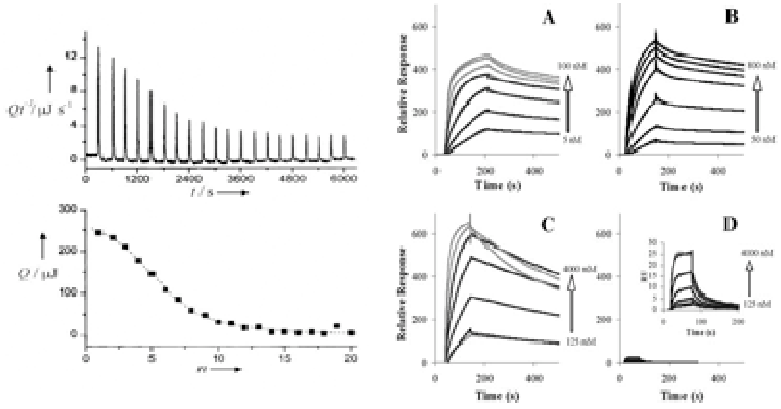

Figure. Interaction between glycosaminoglycans and proteins using ITC and SPR.

<u>X-ray crystallography and NMR spectroscopy to solve the structure of glycosaminoglycan-protein complexes</u>: The atomic contacts between glycosaminoglycan and its binding protein are determined by single crystal X-ray crystallography. The first high-resolution co-crystal structure, bFGF in complex with a heparin tetrasaccharide was published by our laboratory and about half of all published co-crystal structures were done in collaboration with the Linhardt laboratory. More complex crystal structures includes the signal transduction complex, FGF_2-$FGFR_2$-(heparin decasaccharide)$_2$ improves the understanding of growth factor signaling in the signal transduction pathway. The complex with heparin lyase bound to its heparin substrate provides an improved understanding of the mechanism and specificity of this important class of enzymes.

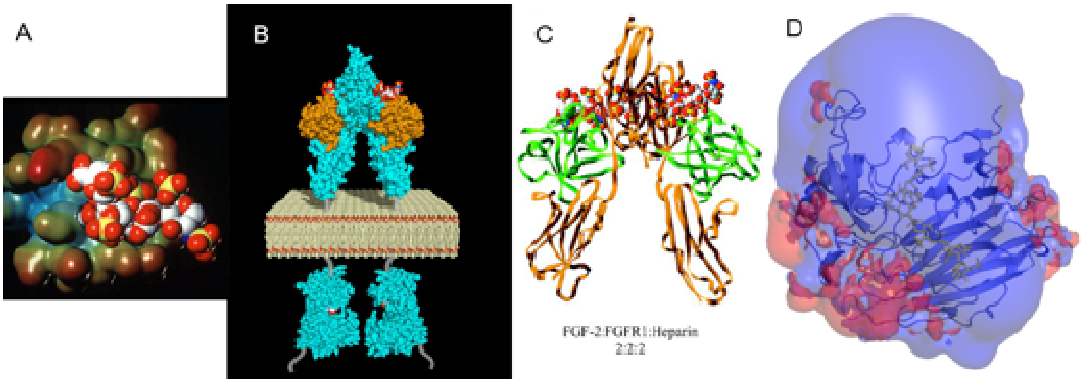

Figure. X-ray co-crystal structures. A. bFGF-heparin tetrasaccharide, B. Model of signal transduction complex, FGF_2-$FGFR_2$-(heparin decasaccharide)$_2$ C. FGF_2-$FGFR_2$-(heparin decasaccharide)$_2$ X-ray structure, and D. heparin lyase structure with bound heparin with surrounding charge field.

NMR solution structures of heparin-protein complexes are currently being solved of chemokines and growth factors in complex with heparin and heparin oligosaccharides.

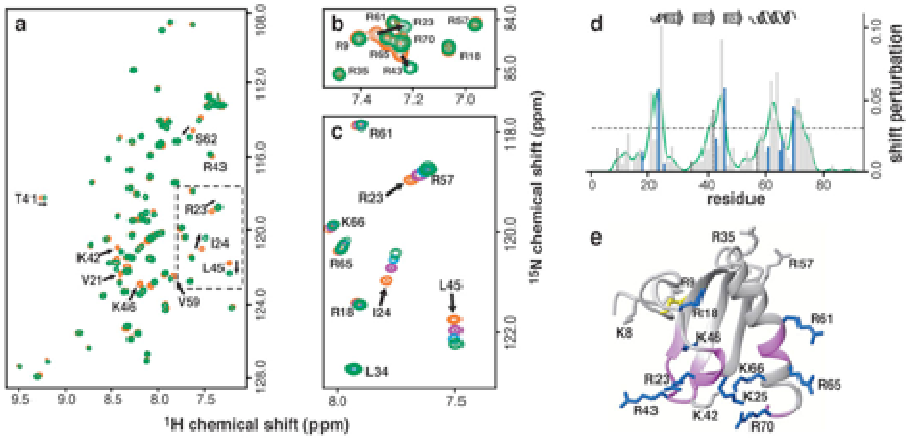

Figure. A 2D-shift map of a heparin chemokine complex showing atomic contacts between binding partners.