Structural Characterization of the DC-SIGN-Lewis^X Complex

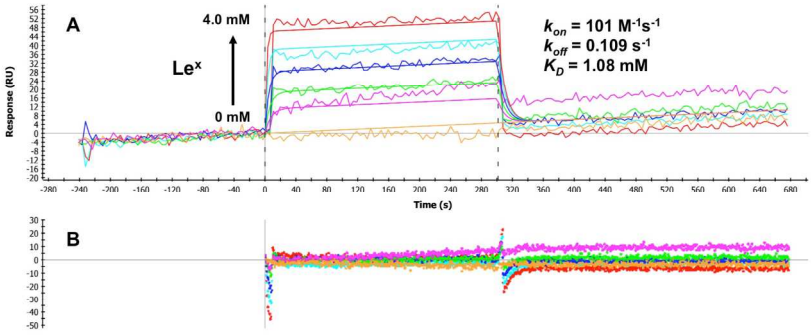
Kari Pederson[1], Daniel A. Mitchell[2], James H. Prestegard[1]*

[1]Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602

[2]Warwick Medical School, University of Warwick, Coventry, England, U.K.

Surface Plasmon Resonance. Surface plasmon resonance was performed using the Bio-Rad ProteOn XPR36 instrument and high ligand density GLH sensor chips. All sensorgrams were recorded at 25˚C at a flow rate of 25 µl/ml in 25mM Tris pH 7.5, 100mM NaCl, 2.5mM $CaCl_2$. Soluble recombinant DC-SIGN tetramer was immobilized at high density (20,000 response units) on activated GLH sensor chips via amine coupling with sulfo-N-hydroxysuccinimide and 1-Ethyl-3-(3- dimethylaminopropyl)carbodiimide and excess reactive groups were blocked using 1M ethanolamine pH 8.0. Lewis^x trisaccharide in solution phase was flowed over the immobilized DC-SIGN at concentrations of 0, 0.25, 0.5, 1, 2 and 4mM and sensorgrams recorded direct binding in high sensitivity mode. Sensorgram data, corrected via baseline subtraction of a blank channel run in parallel, were subsequently processed using Langmuir kinetics modeling (correcting for baseline drift) to derive values for k_{on}, k_{off} and K_D.

Figure S1. Kinetic analysis of Le^x interactions with DC-SIGN tetramer. Sensorgrams measured from solution-phase Le^x flowed over immobilized DC-SIGN tetramer at the range of concentrations indicated on the left (0, 0.5, 1, 2 and 4mM). Smooth lines indicate theoretical curves according to the 1:1 Langmuir algorithm. Parameters are indicated on the right (k_{on} = 101$M^{-1}s^{-1}$; k_{off} = 0.109s^{-1}; K_D = 1.08mM). Panel B shows the residuals derived from Langmuir modeling. The kinetic constants deviate significantly from those observed in solution, likely because of surface effects. Only the dissociation constant was used in support of STD modeling.



180x135mm (300 x 300 DPI)