Supplementary Information

Experimental Section

1. Chemicals and materials

1-ethyl-3-[3-dimethyl-aminopropyl]carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (sulfo-NHS) and ethanolamine (1 M, pH 8.5) were obtained from Attana, Sweden. Concanavalin A (Con A) and the blue nucleic acid stain Heochst 33258 were purchased from Sigma. Wheat germ agglutinin (WGA), Peanut Agglutinin (PNA), Ulex Europaeus Agglutinin I (UEA-I) and Soybean Agglutinin (SBA) were purchased from Vector Laboratories. N-Acetylglucosamine (GlcNAc) and N-Acetylgalactosamine (GalNAc) was purchased from Sigma Methyl-β-D-galactopyranoside was purchased from Tokyo Chemical Industry Co. Ltd. L-Fucose was purchased from Energy Chemical. Jurkat cells and K562 cells were kindly provided by Prof.Bin Gao in the Institute of Microbiology of the Chinese Academy of Sciences (IMCAS). All biosensor experiments were undertaken using an Attana Cell A200 QCM instrument (Attana AB, Stockholm, Sweden) at 22 ^{o}C and the running buffer was phosphate buffered saline (PBS) at pH 7.2.

2. Cell culture and preparation of cell suspension

Human acute lymphocytic leukemia (ALL) cell line Jurkat and Human acute myelocytic leukemia cell line K562 were cultured in RPMI 1640 medium (GIBCO), supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin. All the cell lines were cultured at 37 ^{o}C with 5% CO_2 and 95% humidity and were maintained by replacement of the medium every 2-3 days to keep the cell density between 1×10^5 and 1×10^6 cells/mL. To prepare the cell suspension for cell capture, the medium was removed by centrifugation and the cells were washed with 5 mL PBS. The cell pellets were then resuspended using appropriate volume of PBS to obtain the cell suspension at a concentration of 2×10^6 cells/mL.

3. Capture suspension cells

First, Con A was immobilized on the Attana LNB-Carboxyl Sensor Chip surface using amine coupling procedure. Briefly, the Attana LNB-Carboxyl Sensor Chip surface was inserted into the biosensor system and allowed to stabilize in phosphate buffered saline (PBS, pH 7.2) running buffer at a flow rate of 100 µL/min. When the baseline was stable, the flow rate was lowered to 10 µL/min. For activation of the surface, a fresh reagent mixture containing 0.2 M EDC and 0.05 M sulfo-NHS was injected twice times for a period of 300 s each. Subsequent to surface activation, Con A diluted in 10 mM acetic acid buffer, pH 4.5 at a concentration of 100 µg/mL was injected several times over the surface for 300 s each to saturate the surface. To deactivate remaining sulfo-NHS esters on the surface, two injections of 1 M ethanolamine pH 8.5 was performed with a contact time of 300 s each. Then the sensor chip was withdrawn from the instrument and ready for cell capture. A drop of 50 µL cell suspension containing 100 000 cells (2×10^6 cells/mL in PBS) was added onto the sensor surface and incubated for 20 min at room temperature. Following incubation, the sensor surface was washed 3 times with PBS to remove uncaptured cells. To evaluate the cell coverage on the sensor surface, the nuclei were stained with Hoechst 33258 and visualised under a fluorescent microscopy (Olympus BX53). Then, the sensor chip can be docked into an Attana Cell A200 QCM instrument and a biosensor experiment measuring the interactions between lectins and the cell surface can be started.

4. QCM analysis of lectin–cell interactions

The sensor chip was fitted to the instrument and allowed to stabilize under a continuous flow (20 µL/min) of running buffer. The measurements were initiated when the resonant frequency was stable (baseline drift < 0.2 Hz/min). Lectins were prepared in running buffer and injected over the surface of the chips at a range of

concentrations, allowed to bind for 105 s and dissociate for 295 s. The resonant frequency of the quartz crystal and the frequency shift (Δf) associated with binding events or dissociation were recorded with the Attester software (Attana, Sweden) in real time. Following each association and dissociation cycle, the cell chips were regenerated between measurements by an injection of corresponding monosaccharides at high concentration (300 mM), and immediately re-equilibrated with running buffer. The glycosylation of Jurkat and K562 cell surface was evaluated by measuring the binding properties of a range of diverse lectins.

5. Competitive inhibition

Competition experiments were performed to evaluate the specificity of WGA binding to Jurkat cells. A mixture solution containing 50 μg/mL WGA and consecutively diluted concentrations of GlcNAc (0.25 - 495 mM) was injected over the suspension cell sensor surface. A corresponding concentration of GlcNAc, without WGA, was injected prior to each measurement and was subtracted from the competitive binding curve as blank. The maximal frequency shift monitored at the end of the association phase (after referencing with the GlcNAc-containing solution) was used to calculate the binding of WGA at each GlcNAc concentration. The observed reduction in measured frequency, compared to injections with pure WGA, was taken as a measure of the competitive effect of the tested GlcNAc. The quantitative binding was then plotted against logarithmic concentration of the GlcNAc and subjected to non-linear regression analysis (GraphPad) using a dose-response inhibition model (equation 1), where Δf_{min} is the minimum frequency difference, Δf_{max} is the maximum frequency difference, and C is the competitor concentration. EC_{50} value (50% competition of WGA binding) was obtained.

$$\Delta f = \Delta f_{min} + \left(\frac{\Delta f_{max} - \Delta f_{min}}{1 + 10^{(C - \log EC_{50})}} \right) \quad (1)$$

2.6 Kinetic studies

The kinetic parameters of the interaction between lectins and cell surface were evaluated by injecting various concentrations of lectins over the sensor surface with regeneration steps in between. The binding curves obtained were analyzed using Evaluation software (Attana), which interprets the kinetics of a reaction by fitting theoretical models based on hypothetical mechanisms. A 1:1 interaction model (with or without a mass transport limitation) was used to global fit the experimental data sets and the kinetic rate constants, such as association rate constant (k_{on}) and dissociation rate constant (k_{off}), can be obtained. From these values, the affinity constant (K_D) was calculated as follows: $K_D = k_{off} / k_{on}$.