



# Characterisation of complex lectin-cell kinetics

## OBJECTIVE

Determine the binding properties of the lectin Concanavalin A (Con A) towards human epidermoid carcinoma cells (A431).

## CONCLUSIONS

- A kinetic approach may be used to determine the apparent affinity as well as rate constants of lectin-cell glycan interactions, providing a greater understanding of the mechanism involved in the recognition of cancer cells by lectins.
- Real time measurements of lectin interactions in both pure and cellular contexts provide a complete characterisation of the binding properties of a carbohydrate-binding protein.
- Attana Cell™ 200 is a robust and accurate tool for evaluation of the binding properties in a biologically relevant environment.



## BACKGROUND

In the development process of new pharmaceutical compounds, kinetic experiments provide insights into potential drug candidates while also defining lead targets. Here we demonstrate the ability of the Attana Cell™ 200 to provide a complete and highly relevant analysis of a protein's binding properties by characterising the interaction both with purified target and in a biological context (cells).

## ATTANA CELL™ 200 BIOSENSOR

The Attana Cell™ 200 system is characterised by the ability to study molecular interactions with cells grown directly on the sensor surface. Even higher biological relevance is attained through features such as continuous flow, physiological temperatures and label-free detection. High data quality is achieved by direct measurements in real-time, avoiding disturbances caused by secondary detection.

## METHOD

**Preparation of Cell sensor chip:** A431 cells were seeded onto the cell compatible sensor surface and placed at 37°C with 5% CO<sub>2</sub> for 24h. Cells were subsequently fixed in 3.7% formaldehyde and inserted into the Attana Cell™ 200 biosensor for stabilisation in running buffer (PBS pH 7.4 supplemented with 0.025% Tween® 20) at a flow rate of 25 µl/min.

**Preparation of mannan surface:** Mannan was immobilised on an Attana carboxyl chip using aldehyde coupling in HEPES buffer at a flow rate of 10µl/min.

All experiments were performed at a flow rate of 25 µl/min. Con A was prepared at appropriate concentrations in running buffer. The evaluation of the binding was performed in real time by monitoring the association and the dissociation phases as depicted on the diagram in Figure 1. The surface was regenerated using glycine 10 mM pH 1.0 supplemented with NaCl 0.5 M, before a new binding curve was recorded. Reproducibility and efficiency of the regeneration were tested by monitoring successive Con A binding curves at the same concentration (with regeneration steps in between injections) (Figure 2). The specificity of the binding of Con A to the immobilised cells was evaluated by fluorescence microscopy (Figure 2).

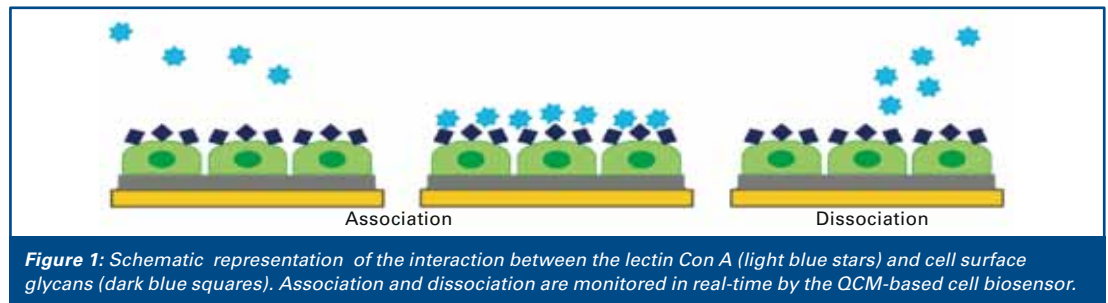
To determine the binding profile of Con A, its binding was monitored at different concentrations on both immobilised carbohydrates and directly on cells. Rate constants of the reaction were then extracted by global fitting of a theoretical 1:1 model by using the Evaluation kinetics software.

## RESULTS

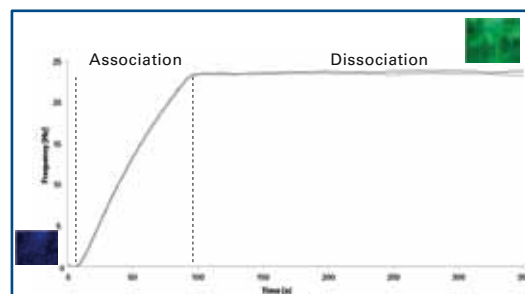
**Specificity/reproducibility:** Con A at a concentration of 25 µg/ml was injected over the sensor surface with immobilised A431 cells, and the binding curves were monitored using the Attester Software. A microscopic evaluation was performed before and after Con A was injected. Figure 2 confirms that the binding monitored on the Attana Cell™ 200 corresponds to the interaction occurring between Con A and cell surface receptors. Two binding events were monitored successively and superimposed in Figure 2, showing a high degree of reproducibility.



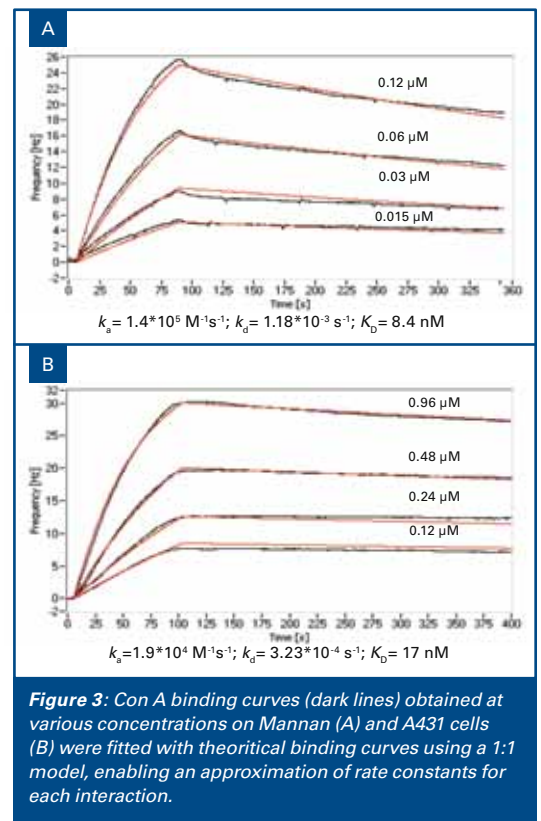
# Characterisation of complex lectin-cell kinetics



**Determination of the complex kinetics of Con A:** In order to understand the binding behaviour of Con A in a biological context, a complex kinetic evaluation was performed. This approach is based on the determination of kinetic parameters both on a surface constituted of immobilised mannan and on a cell sensor chip. Several injections of Con A at different concentrations were performed on each surface and the corresponding binding curves were monitored. Rate constants were determined by global fitting of a theoretical model using the Evaluation Software (Figure 3). The overall affinity of Con A slightly differs according to the surface it interacts with, ( $K_D$  mannan= 8.4 nM  $K_D$  Cells= 17 nM). More importantly, high discrepancy is observed in both association ( $k_a$  mannan=  $1.4 \cdot 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ;  $k_a$  cells=  $1.9 \cdot 10^4 \text{ M}^{-1} \text{ s}^{-1}$ ) and dissociation ( $k_d$  mannan=  $1.18 \cdot 10^{-3} \text{ s}^{-1}$ ;  $k_d$  cells=  $3.23 \cdot 10^{-4} \text{ s}^{-1}$ ) rate constants suggesting a difference in target accessibility and a probable higher contribution of avidity/rebinding in a cellular environment. The biologically relevant cellular environment suggests that this kinetics data will provide the sounder base for selection decisions.



**Figure 2:** Duplicates showing the reproducibility of binding curves for the interaction between Con A and A431 cells. Microscopic verification of the specificity of the binding was also performed. The presence of cells was verified before injection of Con A by staining nuclei (blue). After injection of the FITC-Con A the microscopic observation shows a high binding of the lectin to the cells (green).



**Figure 3:** Con A binding curves (dark lines) obtained at various concentrations on Mannan (A) and A431 cells (B) were fitted with theoretical binding curves using a 1:1 model, enabling an approximation of rate constants for each interaction.

Attana Materials	Item Code
Amine Coupling Kit	3501-3001
Attana® LNB Carboxyl Sensor Chip	3623-3033 (pack of 3) 3623-3103 (pack of 10)
Attana Cell™ 200	3745-3001
Attana MPT 1 Cell sensor chip	3621-3103
Attaché 2.0 software suite	3470-3001

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