

Competition Study of Protein - Carbohydrate Interactions

Zhichao Pei, Henrik Anderson, Teodor Aastrup, Olof Ramström

Prepared in cooperation with the Department of Chemistry, Royal Institute of Technology, Sweden.

Introduction

Interactions between carbohydrates and proteins are involved in numerous cellular processes, for instance bacterial adherence to host tissue.

Understanding carbohydrate - protein binding and consequently, the knowledge of how to manipulate these interactions will be of great value in the development of new drugs.

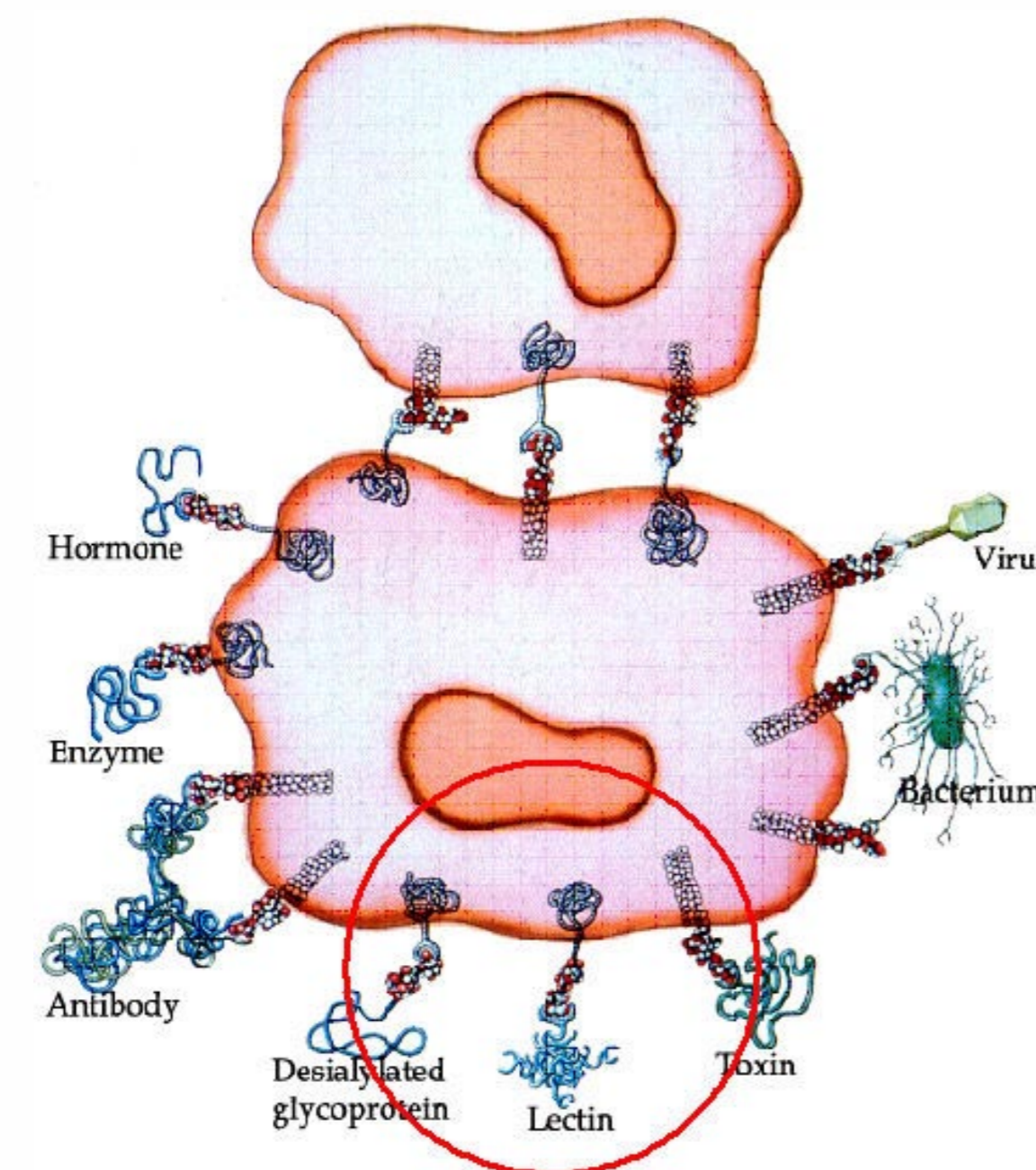


Fig 1. Carbohydrate Interactions © BioCarb AB

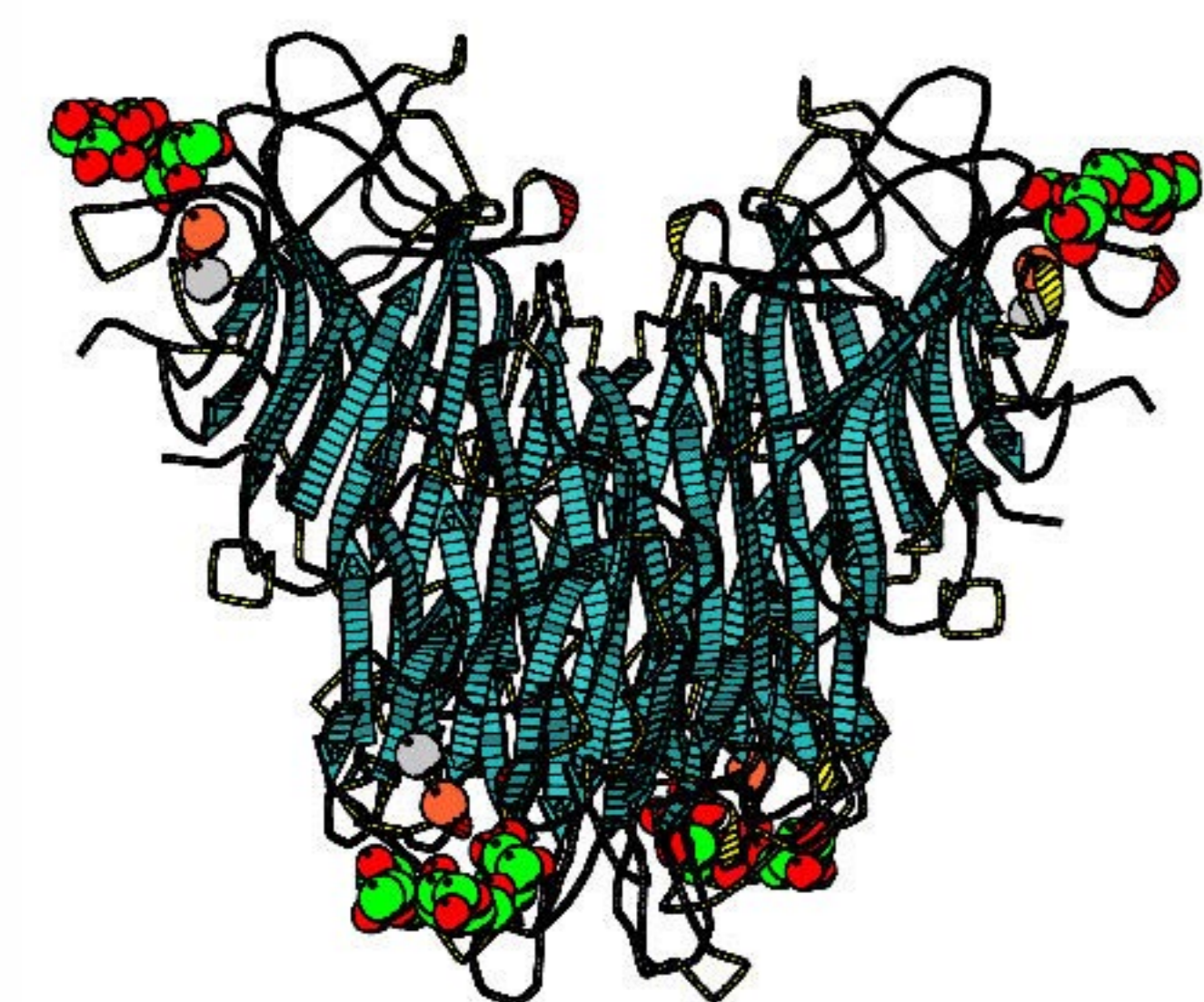


Fig 2. Concanavalin A (Con A) © IMB-Jena Image library

Studies of carbohydrate-protein interactions have previously been performed in micro-titer plate format using solid-phase enzyme linked lectin assays (ELLA).

To facilitate the study of these interactions, a method for label-free measurements has been developed using the Attana 100 biosensor to monitor binding events in real time without labelling.

The method relies on measurement of the inhibition of a well-known carbohydrate-lectin binding pair, Concanavalin A (Con A)-Yeast Mannan, by lectin binding carbohydrates. The binding of Con A to a surface was monitored under the influence of competing carbohydrates.

Equipment

The experiments use an Attana 100 continuous-flow biosensor and polystyrene coated sensor chips.

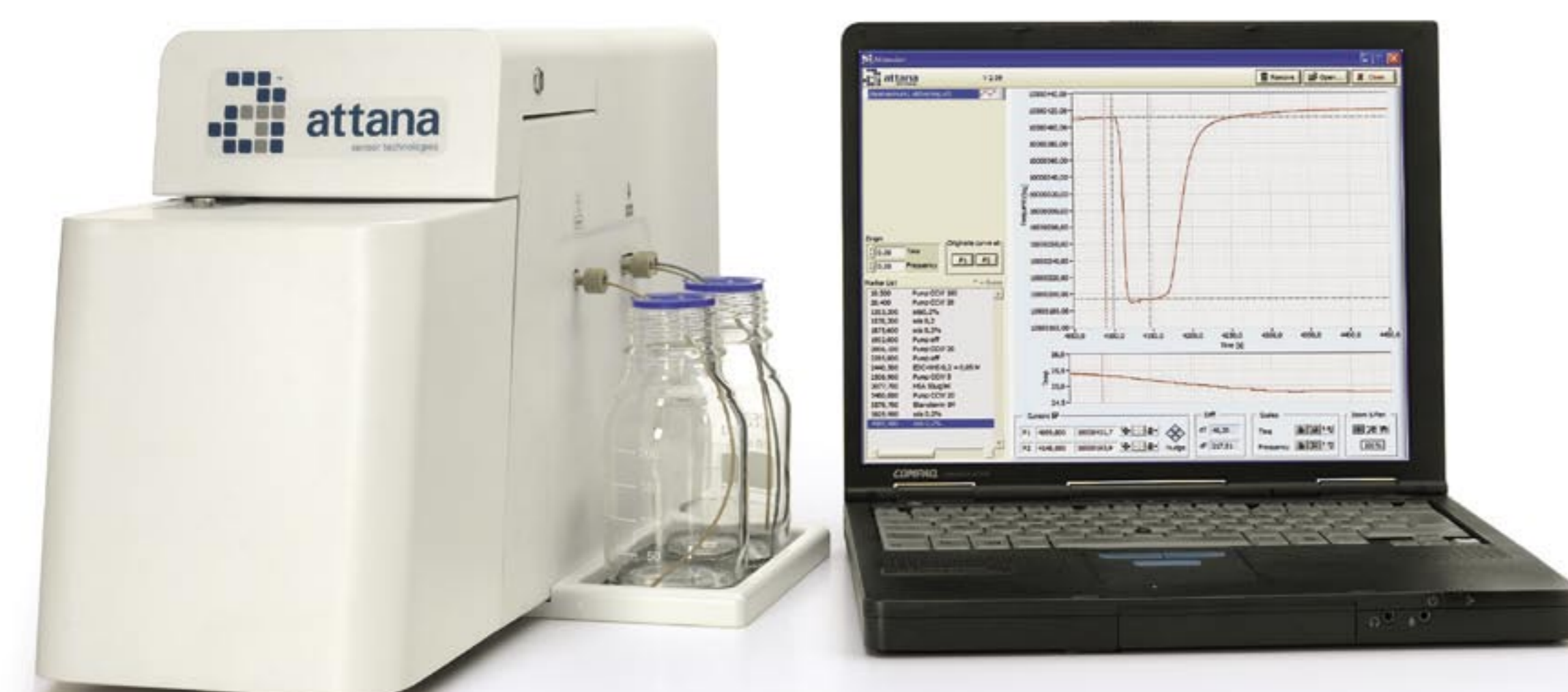


Fig 3. Attana 100 system along with Attester software

Experimental Method

The first step was to immobilize mannan on a polystyrene-coated sensor surface. Next, the surface was blocked with bovine serum albumin (BSA) in repeated injections until the surface was saturated and showed no additional binding. The surface was then ready for screening of Con A binding carbohydrate ligands.

Samples were injected into the biosensor and after the samples had passed the surface, the surface was regenerated with PBS buffer at pH 1.5.

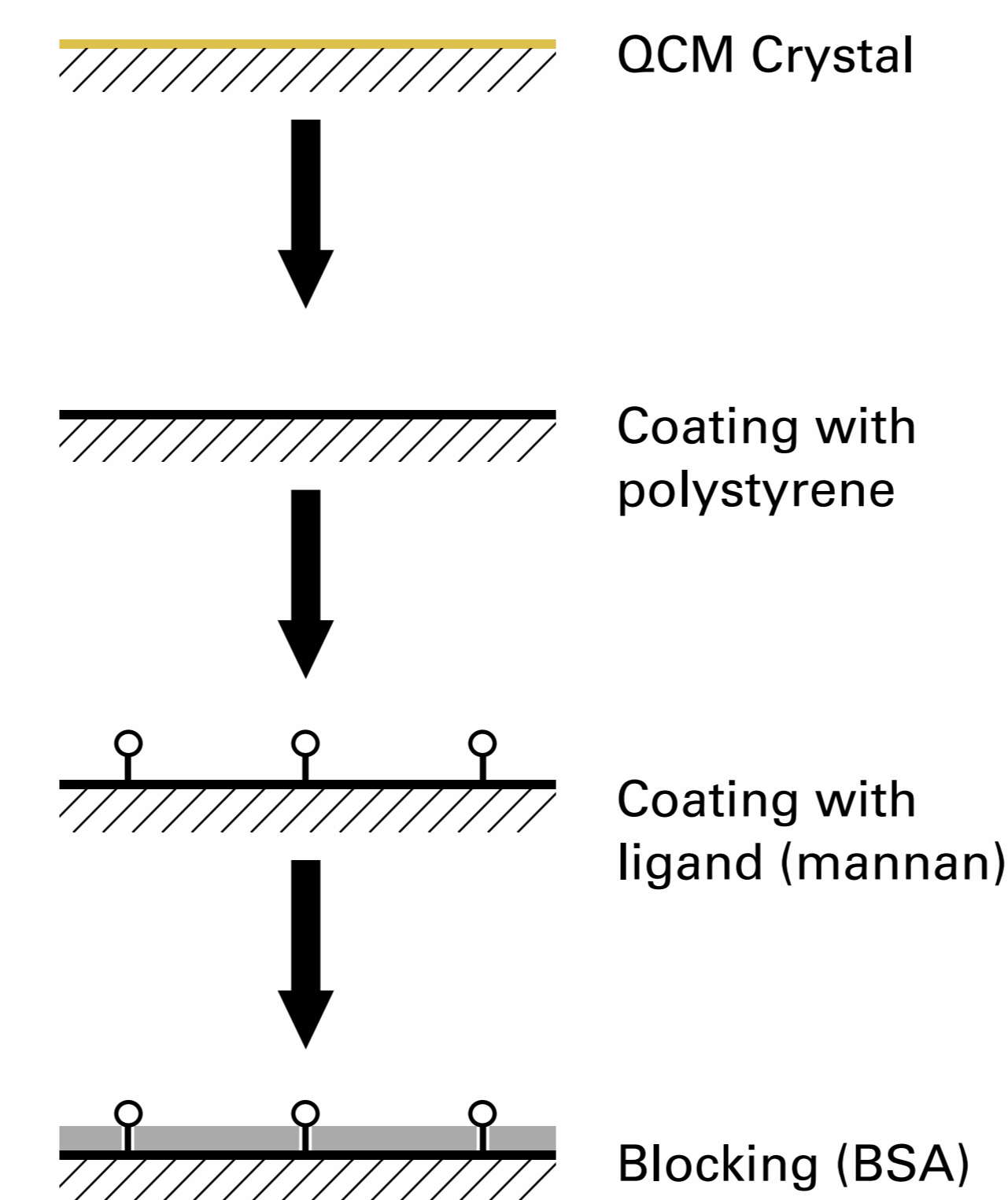


Fig 4. Immobilization scheme

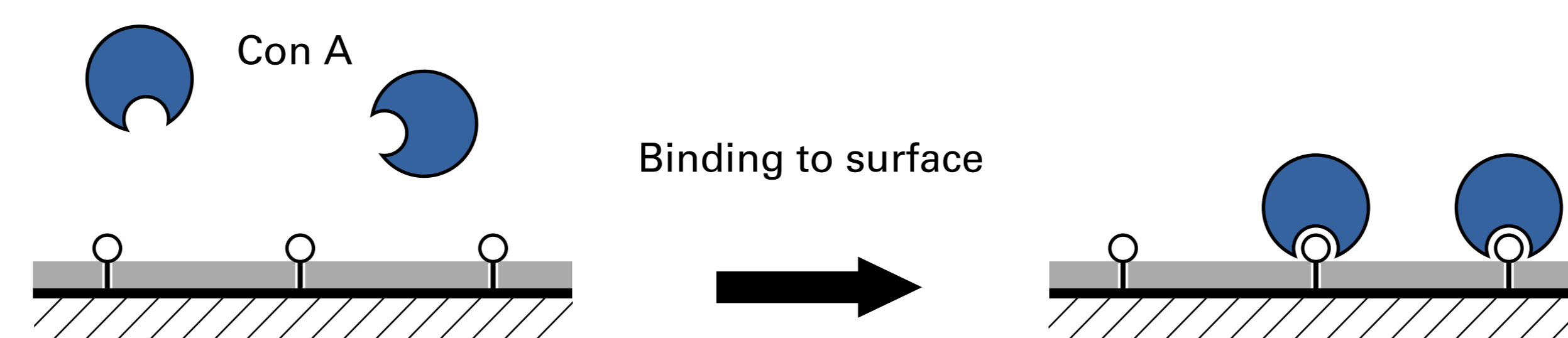


Fig 5. Con A binding to immobilized mannan

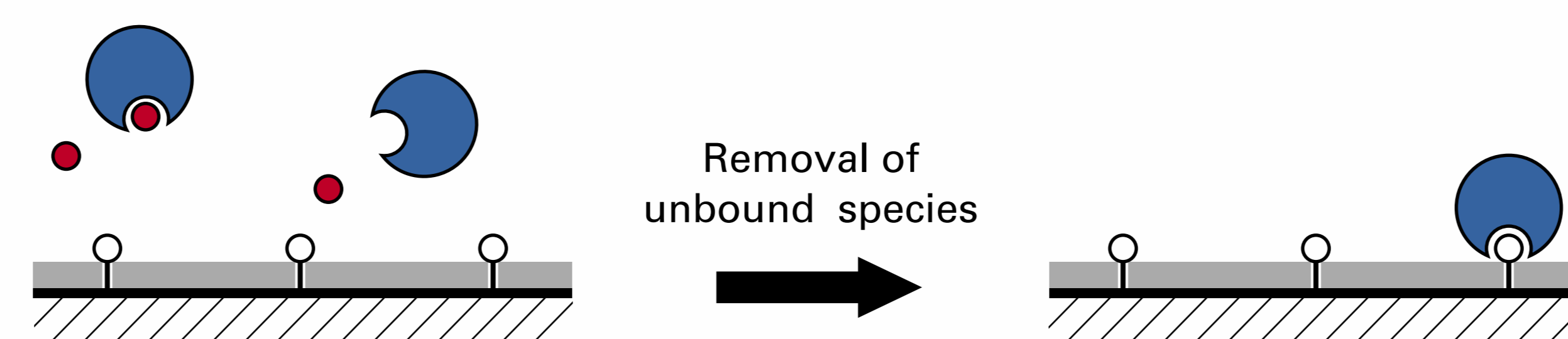


Fig 6. Con A binding to mannan and competitor.

Experimental Results

A typical example of the frequency shift recording from a competition assay is displayed in Fig. 7, where 4-Nitrophenyl- α -D-mannopyranoside was used as competitor for Con A/mannan binding.

Increasing concentrations of competitor yielded gradually less binding of Con A to the surface. The resulting competitive binding curves of all four ligands are displayed in Fig. 8. The competitive binding data were subjected to non-linear regression analysis, and the resulting EC₅₀-values are presented in Table 1. The four competitive carbohydrates showed competition constants ranging from 0.18 to 5.3 mM.

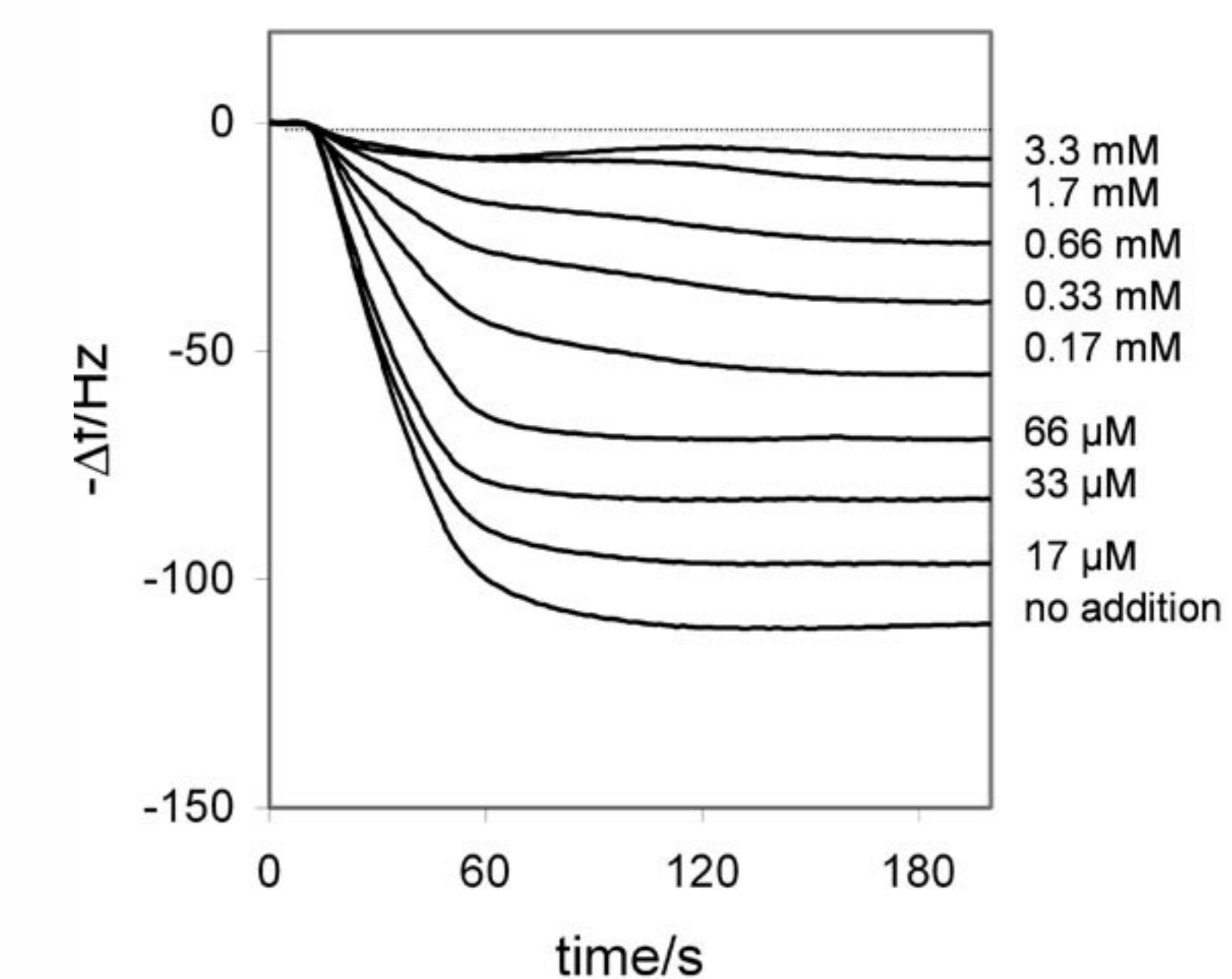


Fig 7. Frequency response plots of the inhibitory effect of competitor on Con A/mannan binding. Increasing concentration of competitor results in reduced frequency response.

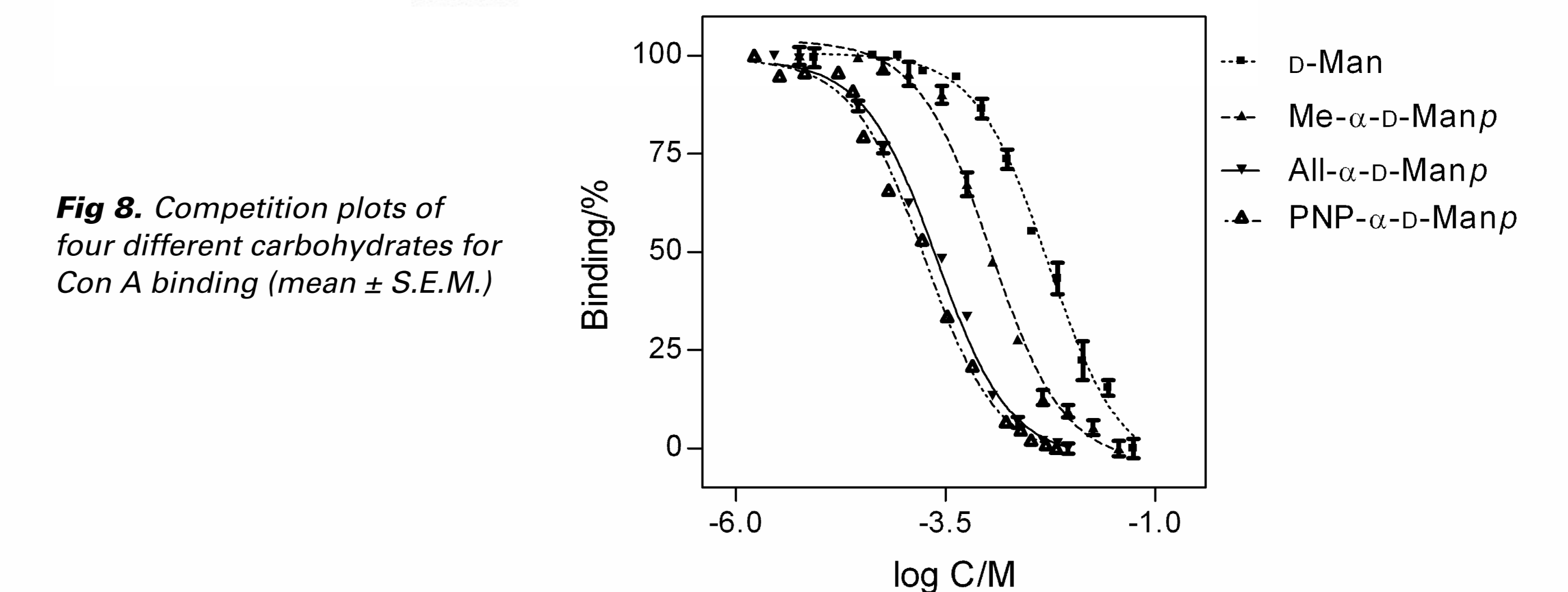


Fig 8. Competition plots of four different carbohydrates for Con A binding (mean \pm S.E.M.)

Ligand	EC ₅₀ (mM) QCM (95% CI)	EC ₅₀ (mM) (ELLA)
D-Mannose (α/β) ^a	5.3 (4.4-6.5)	>2.5
Methyl- α -D-mannopyranoside	1.1 (0.90-1.3)	0.92
Allyl- α -D-mannopyranoside	0.25 (0.21-0.29)	0.26
4-Nitrophenyl- α -D-mannopyranoside	0.18 (0.15-0.21)	0.11

Table 1. EC₅₀-values: QCM vs. ELLA

Conclusions

- The developed assay provides a method for screening the binding of carbohydrate ligands to lectins.
- In contrast to ELLA based approaches, this method requires no labeling of the lectin and the data is delivered in real time.
- Assay data for four model compounds have been compared to literature data and have been found to be in good agreement with previous studies.

Telephone: +46 8 410 200 00
E-mail: info@attana.com
www.attana.com



attana
sensor technologies