## Supplementary Material

### Immobilization of lectins onto silica

Nucleosil Si-300 silica was first reacted with an epoxy-containing silane and then converted into a diol form, as described previously [1]. For this process, 1.0 g of silica was suspended in 8.5 mL of 0.10 M acetate buffer (pH 5.5) and sonicated for 5 min under vacuum. Next, a 200 μL portion of (3-glycidoxypropyl)trimethoxysilane was added, and the resulting solution was stirred for 5 h at 90 ºC. The epoxy-derivatized silica was washed three times with 10 mL water and four times with 10 mL of a dilute sulfuric acid solution (pH 3.0). The epoxy groups were converted into diols by suspending the silanized silica in 150 mL of the pH 3.0 sulfuric acid solution and refluxing for 1 h. The diol silica was washed three times with 10 mL water and dried plus stored in a 50 ºC oven until further use.

The diol silica was next converted into aldehyde-activated silica [2]. For this process, a 0.2 g portion of diol silica was suspended in 4 mL of 90% acetic acid that contained 0.2 g periodic acid. This solution was sonicated for 5 min under vacuum, and the mixture was shaken in the dark for 2 h. The silica was washed six times with 3 mL water to remove the remaining periodic acid, followed by three washes with 3 mL of 0.10 M sodium bicarbonate buffer (pH 8.0) containing 0.50 M sodium chloride [3]. The aldehyde-activated silica was used immediately for immobilization.

The coupling of a lectin to the aldehyde-activated silica was accomplished by using reductive amination (i.e., the Schiff base method) [4]. A 9 mg portion of concanavalin A (Con A) or 2 mg of *Aleuria Aurantia* lectin (AAL) was placed into a 2.5 mL suspension of ~0.2 g

aldehyde-activated silica in the pH 8.0 sodium bicarbonate buffer along with 50 mg of sodium cyanoborohydride. This suspension was placed on a rocking mixer and allowed to react at room temperature for three days. The slurry was then washed three times with 3 mL of 0.10 M sodium bicarbonate buffer (pH 8.0) containing 0.5 M sodium chloride. A 5 mg portion of sodium borohydride was slowly added to this suspension in several portions over 90 min to reduce any remaining aldehyde groups. The slurry was washed three times with 0.10 M sodium bicarbonate buffer (pH 8.0) containing 0.50 M sodium chloride, followed by three washes with the application/loading buffer that was to be used later with this support (see Section 2.5). The slurries containing the lectin supports were stored in the same application buffers at 4 $^{o}$C until use. Control supports were prepared in the same manner but with no lectin being added during the immobilization process.

**Determination of amount of active sites on Con A and AAL microcolumns for small solutes**

Frontal analysis measurements of the binding capacity for a Con A microcolumn was performed by continuously applying a solution containing 1.3 mM *p*-nitrophenyl α-D-mannopyranoside (*p*-NP-α-D-Man) to a 2.1 mm i.d. × 5.0 cm Con A microcolumn at 0.10 mL min$^{-1}$. The column was maintained at 4 $^{o}$C and detection was performed at 400 nm. Similar experiments were performed on a control microcolumn with the same dimensions to correct for non-specific binding and column void time. Triplicate analysis was carried out on each type of microcolumn.

Frontal analysis studies of an AAL microcolumn were performed by continuously

infusing 1.6 mM *p*-nitrophenyl α-L-fucopyranoside (*p*-NP-α-L-Fuc) onto a 2.1 mm i.d. × 5.0 cm AAL microcolumn at 0.10 mL $min^{-1}$. The column was maintained at 25 ºC and detection was performed at 400 nm. Similar experiments were again performed on a control microcolumn with the same dimensions to correct for non-specific binding and column void time, and all measurements were carried out in triplicate.

Typical chromatograms and breakthrough curves that were obtained in these experiments are shown in Figure 1S. It was found that the amount of active binding sites for *p*-NP-α-D-Man in the Con A microcolumn was 89 (± 2) nmol. The amount of binding sites for *p*-NP-α-L-Fuc in the AAL microcolumn was 152 (± 5) nmol.

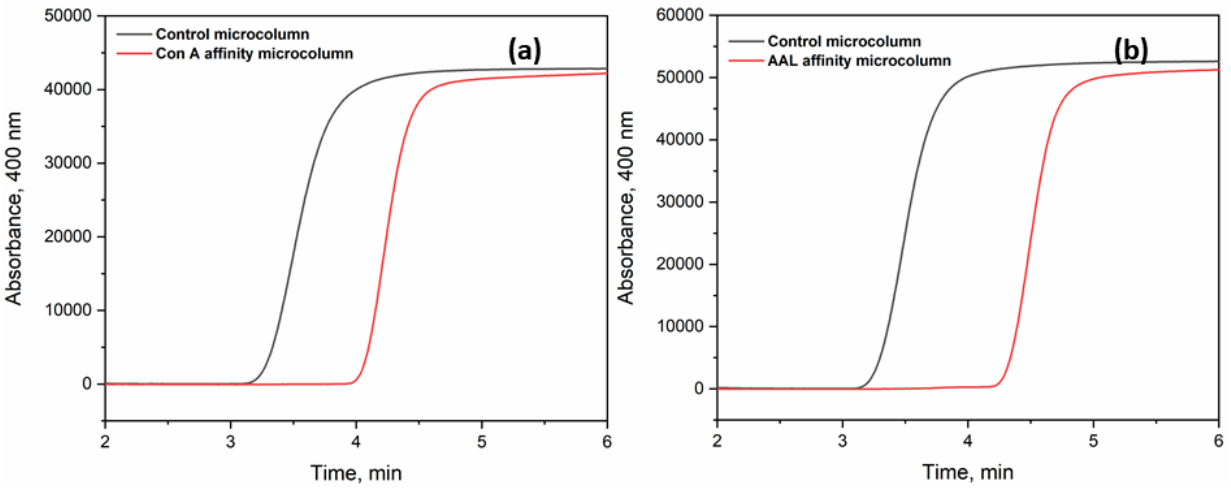


**Figure 1S.** Chromatograms obtained by continuously infusing (a) 1.3 mM *p*-NP-α-D-Man onto a Con A microcolumn and (b) 1.6 mM *p*-NP-α-L-Fuc onto an AAL microcolumn. The conditions for (a) were as follows: flow rate，0.10 mL $min^{-1}$; temperature, 4 ºC; mobile phase, 10 mM Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl, 0.50 mM $CaCl_2$, 0.50 mM $MgCl_2$ and 0.50 mM $MnCl_2$. The conditions for (b) were: flow rate，0.10 mL $min^{-1}$; temperature: 25 ºC; mobile phase, 10 mM Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl.

**The effect of temperature on the retention of AGP glycoforms on a Con A microcolumn**

The retention of AGP glycoforms on a Con A microcolumn was monitored at temperatures spanning from 10 to 50 ºC. A 20 µL portion of a sample containing 5 mg $mL^{-1}$ AGP was injected onto a 2.1 mm i.d. × 5.0 cm Con A microcolumn under isocratic conditions and at a flow rate of 0.05 mL $min^{-1}$. The mobile phase was 10 mM Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl, 0.50 mM $CaCl_2$, 0.50 mM $MgCl_2$ and 0.50 mM $MnCl_2$. Detection was performed at 280 nm.

As is shown in Figure 2S, it was found that an increase in column temperature reduced the retention time of the retained AGP fraction while the retention time of the non-retained AGP fraction remained constant. This is believed to have occurred because there was a change in the equilibrium constant between Con A and the retained AGP glycoforms as the temperature was increased.

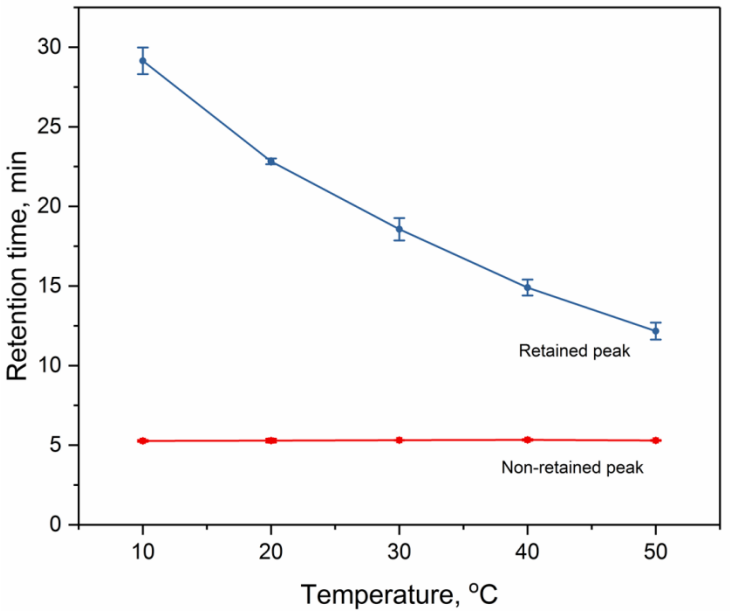


**Figure 2S.** The retention times of non-retained and retained AGP fractions on a 2.1 mm × 5.0 cm Con A microcolumn at various temperatures. The chromatographic conditions were as follows: sample volume, 20 µL; sample load, 100 µg AGP; flow rate, 50 µL $min^{-1}$; mobile phase, 10 mM Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl, 0.50 mM $CaCl_2$, 0.50 mM $MgCl_2$ and 0.50 mM $MnCl_2$. The error bars represent ± 1 S.D. ($n$ = 3).

**Determination of amount of active sites for ovalbumin on a Con A microcolumn**

Frontal analysis of ovalbumin on a Con A microcolumn was performed by continuously infusing a 1.0 mg $mL^{-1}$ solution of ovalbumin onto a 2.1 mm i.d. × 1.0 cm Con A microcolumn at 0.10 ml/min. The column was maintained at 10 ºC, and detection was performed at 280 nm. The application buffer was 10 mM Tris-HCl buffer, pH 7.4, with 0.15 M NaCl, 0.50 mM $MgCl_2$, 0.50 mM $MnCl_2$, 0.50 mM $CaCl_2$. The elution buffer has a similar composition to the application/loading buffer but also contained 0.10 M methyl α-D-mannopyranoside as a competing agent for step elution [3]. Similar experiments were performed on a control microcolumn with the same dimensions as the Con A microcolumn to correct for non-specific binding and the column void time. Triplicate measurements were made on each microcolumn.

After the first derivative was taken for the frontal analysis curve of ovalbumin on a Con A microcolumn, two breakthrough curves were identified (see Figure 3S). It has been reported that ovalbumin can be separated into two fractions by Con A Sepharose at 10 ºC, with the ratio of fraction II vs fraction I being 2:1 and the overall binding affinity being equal to $2 \times 10^5$ $M^{-1}$ at 10 ºC [5]. In this study, the average breakthrough time for ovalbumin was found by using the breakthrough times of the individual curves and the relative measured amount of ovalbumin that made up each curve. Based on this approach, amount of active binding sites for ovalbumin, which was used here as a model glycoprotein, was found to be 6.7 (± 0.6) nmol. This value was 13-fold smaller than the amount of active sites that were measured on the same type of column for *p*-NP-α-D-Man (i.e., 89 nmol). This difference is probably due to the small size of

*p*-NP-α-D-Man vs. ovalbumin, which gave *p*-NP-α-D-Man easier access to binding sites on Con A and less steric hindrance in these interactions (Note: although there are up to four binding sites on Con A for a small target, ovalbumin will have 1:1 to this lectin because the size of ovalbumin will tend to block the additional sites once ovalbumin binds to Con A). Based on comparable size of ovalbumin (42.7 kDa) versus AGP (41-43 kDa) [6,7], the binding capacity for ovalbumin was determined to be a more accurate measure of the binding capacity that would be expected for AGP glycoforms that could also bind to Con A.

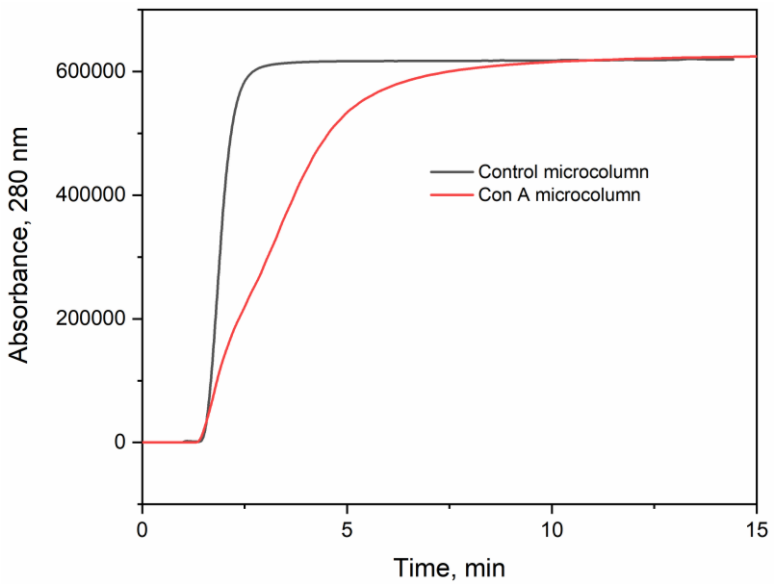


**Figure 3S**. Frontal analysis of ovalbumin on a 2.1 mm i.d. × 1.0 cm Con A microcolumn and a control microcolumn. The conditions were as follows: application/loading buffer: 10 mM Tris-HCl buffer, pH 7.4, with 0.15 M NaCl, 0.50 mM $MgCl_2$, 0.50 mM $MnCl_2$, and 0.50 mM $CaCl_2$; elution buffer, the same composition as the application but also containing 0.10 M methyl α-D-mannopyranoside; flow rate, 0.10 mL $min^{-1}$; column temperature, 10 ºC; detection wavelength, 280 nm. The applied sample contained 1.0 mg $mL^{-1}$ ovalbumin that was dissolved in the application buffer. All frontal analysis studies were performed in triplicate. The time program for the Con A microcolumn was as follows: 0.0-0.5 min, equilibrate with application buffer; 0.5-20 min, continuously apply 1.0 mg mL ovalbumin; 20-35 min, wash column with application buffer; 35-55 min, elute retained ovalbumin at 0.3 mL $min^{-1}$; 55-75 min, regenerate column with application buffer. The time program for the control microcolumn was: 0.0-0.5 min, equilibrating with application buffer; 0.5-20 min, continuously apply 1.0 mg $mL^{-1}$ ovalbumin; 20-35 min, wash column with application buffer.

**Effect of temperature on the binding of AGP glycoforms to an AAL microcolumn**

The area of the retained AGP fraction on an AAL microcolumn was monitored at temperatures spanning from 10 to 50 ºC. A sample concentration of 1.5 mg $mL^{-1}$ AGP was injected onto a 2.1 mm i.d. × 5.0 cm AAL column by using an injection volume of 20 μL. The AAL microcolumn was used with step elution at and a flow rate of 0.75 mL $min^{-1}$. Detection was carried out at 280 nm. The composition of the application/loading buffer was 10 mM Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl. The elution buffer had a similar composition to the loading buffer but also contained 2 mM L-fucose as a competing agent.

As is shown in Figure 4S, it was found that the peak area for the retained AGP fraction was relatively constant between 10 and 50 ºC, with a maximum value being obtained at 30 ºC and a slight decrease of 5.2% or 15.5% being seen at 40 and 50 ºC respectively. This change suggested that there was a slight drop in the activity of AAL towards the AGP glycoforms at the higher column temperatures.

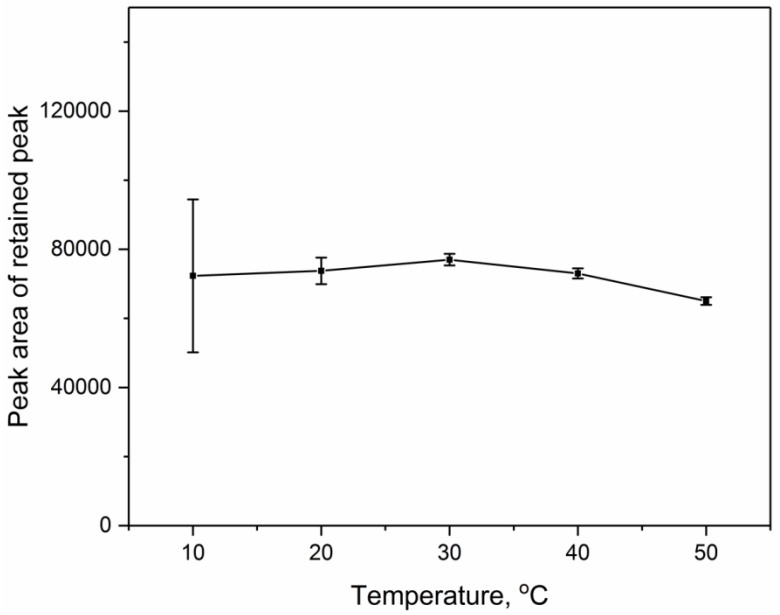


**Figure 4S.** Peak area of the retained AGP fraction on a 2.1 mm × 5.0 cm AAL microcolumn at various temperatures. The error bars represent ± 1 S.D. ($n$ = 3). The conditions were as follows: sample volume, 20 μL; sample load, 30 μg AGP; flow rate, 0.75 mL $min^{-1}$; application/loading buffer, 10 mM Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl; elution buffer, same composition as the application buffer but also containing 2 mM L-fucose.

**References**


[1] R.R. Walters, High-performance affinity chromatography. pore-size effects, J. Chromatogr. A. 249 (1982) 19–28.

[2] G.M. Loudon, Organic Chemistry, 3rd ed., Benjamin Cummings Publishing, Redwood City, CA, 1995.

[3] M. Madera, Y. Mechref, M. V Novotny, Combining lectin microcolumns with high-resolution separation techniques for enrichment of glycoproteins and glycopeptides, Anal. Chem. 77 (2005) 4081–4090.

[4] W.S. Emerson, The preparation of amines by reductive alkylation, Org. React. 4 (1948) 174–255.

[5] V. Shepherd, R. Montgomery, Interaction of ovalbumin and its asparaginyl-carbohydrate fractions with concanavalin A, Biochim. Biophys. Acta - Protein Struct. 535 (1978) 356–369.

[6] A.D. Nisbet, R.H. Saundry, A.J.G. Moir, L.A. Fothergill, J.E. Fothergill, The complete amino-acid sequence of hen ovalbumin, Eur. J. Biochem. 115 (1981) 335–345.

[7] T. Fournier, N. Medjoubi, D. Porquet, Alpha-1-acid glycoprotein, Biochim. Biophys. Acta. 1482 (2000) 157–171.