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Use of Boolean and fuzzy logics in lactose glycocluster research†

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Fuzzy logic systems can be exploited for defining the degrees of true or false binding between calcium mediated multivalent lactose and peanut agglutinin lectin, which are difficult to define with Boolean logic.

Carbohydrate–protein interactions (CPIs) play a crucial role in many biological events, such as cell–cell adhesion, proliferation, bacterial and viral infections.[1] Since CPIs in normal and malignant cells differ significantly, it is crucial to understand them both qualitatively and quantitatively.[2] A variety of techniques, such as surface plasmon resonance (SPR), microarray, quartz crystal microbalance (QCM), and enzyme-linked immunosorbent assay (ELISA),[3] were used for analyzing these interactions. All methods require expensive instruments, laborious experiments and extensive technical expertise. Recently, Boolean logic (BL)[4] was used for real-time and straightforward analysis of CPIs to select the best scaffolds for specific interaction and also for sensing processes.[5] Although the BL model is simple and very effective in differentiating true and false interactions, it is not adequate for describing fine-tuned systems and degrees of truthfulness. The fuzzy logic system (FLS) is a superset of BL extended to characterize partially true values between completely true (1) and completely false (0). It is one of the automatic fine-tuning control systems that can handle several middle steps and define degrees of truth. It stems from the notion that human reasoning and decision making is too complex to be precisely defined.

We have applied BL and FL for optimization of CPIs mediated by Ca^{2+} ions, with emphasis on the multivalent ionic interactions between lactose, appended on a β-cyclodextrin skeleton, and peanut agglutinin (PNA) lectin. We have compared the two methods and concluded that the logic operation of FL is more appropriate for analyzing CPI than BL.

The experimental setup was based on a newly synthesized lactose-modified β-cyclodextrin (**1**) and the two glycodendrimers (**2** and **3**), which were prepared by exploiting the tendency of adamantane and benzene to form stable complexes with β-cylodextrin and its derivatives used as the 'hosts' in complexes **2** and **3** with compounds **4** and **5**, respectively, used as the 'guests' (Fig. 1A). The syntheses of compounds **1** and **2** were described previously.[6] Compound **4** was prepared by reacting the dichloride of ferrocene dicarboxylic acid, prepared by reaction with oxalyl chloride, and adamantyl 2′-amino-β-alanine amide (Fig. 1B). Complexes **2** and **3** include guest molecules having azo and ferrocene templates. The glycodendrimers were used as active components for the performance of logic operations and for construction of truth tables based on chemical inputs. PNA lectin, which recognizes lactose specifically,[7] and calcium ions,

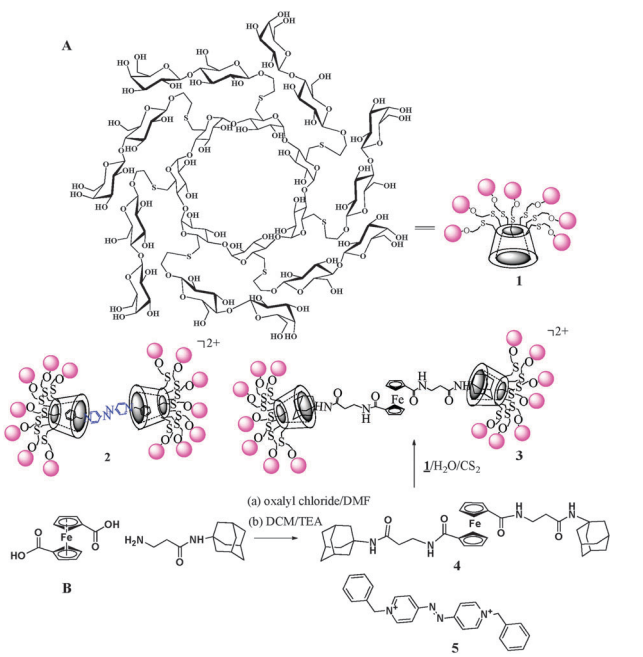

Fig. 1 (A) Structures of lactose glycodendrimers (**2** and **3**); (B) schematic diagram of the mechanism of interactions.

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which interact with lactose, were used as input signals.[8] Isothermal titration calorimetry (ITC), used to determine the thermodynamic parameters of interactions in solution, was utilized for measuring the association constants between PNA lectin and compounds **2** and **3** that were used as output.

For demonstrating the use of the operation AND gate in Boolean logic, PNA lectin and Ca^{2+} ions, separately or in a mixture, were titrated with the glycoclusters **2** and **3** and the process was followed by ITC. The binding processes are accompanied by release of heat and typical isotherms were recorded. Based on the qualitative assessment, the isotherms were fitted to a model containing one set of binding sites. '*N*' values represent the number of carbohydrates on the glycoclusters that are available for binding to PNA lectin or to the Ca^{2+} ions and '*K*' represents the formation constants. (Table 1) For complex **2**, the best fit was obtained for '*N*' values of $\approx$21 and $\approx$232 for Ca^{2+} and PNA, respectively. Since the CCIs are weak, addition of Ca^{2+} ions resulted in weak binding affinity (115 M^{-1}) (Fig. 2a). For PNA lectin interactions, a significant increase in the binding affinity of **2** as compared to CCIs (3.18×10^6 M^{-1}) was observed (Fig. 2b). In the presence of both, Ca^{2+} ions and PNA lectin, the binding affinity was increased, '*N*' value $\approx$334 (Fig. 2c), indicating that Ca^{2+} ions not only assist lectin amino acids in positioning for achieving maximum binding, but also induce inter/intra CCIs and increase the number of binding sites. By setting the binding affinity of CPIs in the presence of Ca^{2+} ions as the threshold level (red line Fig. 3a), **2** exhibited the behavior of the AND logic; in the presence of a single input signal ((1.0) and (0.1)), **2** displayed a low output or no binding at all (0) while with both inputs high

Table 1 Thermodynamic parameters of *cis–trans* isomers in **2** measured by isothermal titration calorimetry; $N = 1/n$

Ligand	n	N	Binding constant (M^{-1})	ΔH (Kcal mol^{-1}) 10^6
2 & Ca^{2+}	$\approx$0.047	$\approx$21	115 $\pm$ 23.0	-3.43 ± 0.81
2 & PNA	$\approx$0.0043	$\approx$232	$3.18 \pm 0.79 \times 10^6$	-0.71 ± 0.005
2 & Ca^{2+} & PNA	$\approx$0.003	$\approx$334	$9.12 \pm 4.87 \times 10^6$	-0.37 ± 0.002
3 & Ca^{2+}	$\approx$0.041	$\approx$24	178 $\pm$ 21.7	-1.79 ± 0.9
3 & PNA	$\approx$0.005	$\approx$200	$2.59 \pm 0.37 \times 10^6$	-0.768 ± 0.009
3 & Ca^{2+} & PNA	$\approx$0.002	$\approx$500	$13.7 \pm 1.13 \times 10^6$	-0.32 ± 0.027

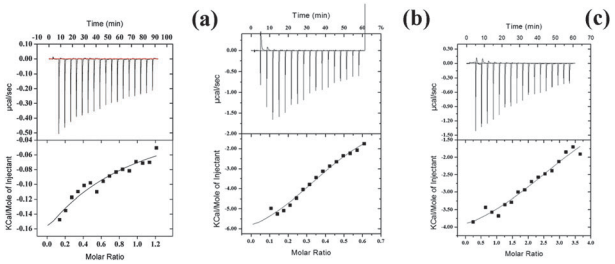
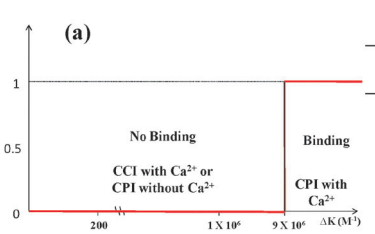


Fig. 2 ITC profile for **2** in H_2O with $CaCl_2$, PNA and both, PNA + $CaCl_2$ at 298 K. Top panels represent the energy (μcal s^{-1}) required to maintain isothermal conditions with respect to the reference cells and lower panels represent the heat evolved from each injection per mole of Ca^{2+} *versus* the molar ratio of (a) conc of **2** = 0.05 mM and $CaCl_2$ solution = 20 mM; (b) conc of **2** = 0.05 mM and PNA = 0.01 mM (c) conc of **2** = 0.05 mM, $CaCl_2$ = 20 mM PNA = 0.01 mM in phosphate buffer pH 7.4.



(b)

(Ca^{2+}, lectin)	2 (BL)	3 (BL)
(0,0)	0	0
(1,0)	0	0
(0,1)	0	0
(1,1)	1	1

Fig. 3 (a) Boolean logic: if binding affinity $\geq 9 \times 10^6$ M^{-1}, it is specific CPI (1 or true) and if binding affinity $\leq 9 \times 10^6$, it is not specific CPI (0 or false). (b) Truth table.

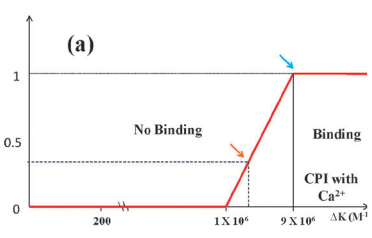
(1, 1) the output is high and represents binding (1). The truth table is presented in Fig. 3b. A major limiting feature of BL is that the values 0 and 1 are mutually exclusive and it is not possible to define a transition from one state to the other, *i.e.*, 'non-binding' to 'binding', by considering a single value. This limitation is overcome by using the fuzzy logic systems (FLSs) for presentation.

FLS can be defined as the nonlinear mapping of an input dataset to a scalar output data. The parameters obtained from the ITC measurements, which reflect the degrees of interaction, were used for the construction of a fuzzy subset. They have degrees of membership ranging between 0 and 1. A subset of 'strong binding', defined in the following way, was used as the 'rule base'.

Strong binding (x) = {0, if $\Delta K \leq 1 \times 10^6$ M^{-1}; 0–1 if $1 \times 10^6 < \Delta K \leq 9 \times 10^6$ M^{-1}, 1 if $\Delta K \geq 9 \times 10^6$ M^{-1}}

Based on this definition we have built the truth table (Fig. 4b) from which we conclude that PNA lectin binding to **2** is '35% strong binding'. Similarly, from the experiment with **3** we received the value 0.28, which is also found to be 'strong binding'.

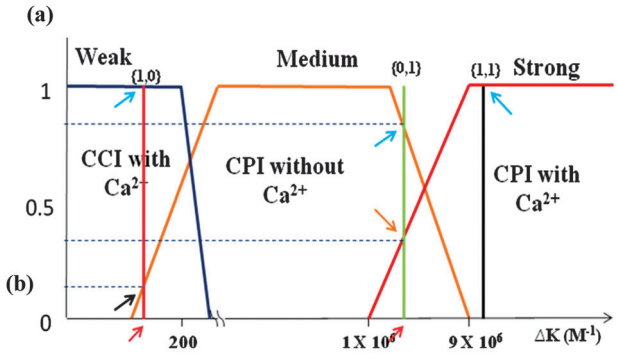
From the above presentation it seems clear what the statement 'degree of strong binding' means. In order to interpret 'weak', 'medium' and 'strong' binding affinities in fuzzy linguistic terms, we have performed a set of operations (union, intersection and complement). 'Intersection' and 'union' are defined as minimum and maximum of two interactions and 'complement' is defined as the negation of specific interaction. Using these operations, fuzzy sets (weak, medium and strong binding) are constructed in which 'weak binding' is defined for systems of binding affinity (x) = {1 (FL) or 0 (BL), if (ΔK) ≤ 200 M^{-1} and gradient binding (0–1) (FW) between $200 \geq \Delta K \leq 250$ M^{-1}}. 'Medium binding' affinity is defined as (y) = {0–1, if $100 \geq \Delta K \leq 250$ M^{-1} (SM) or $1 \times 10^6 \geq \Delta K \leq 9 \times 10^6$ M^{-1}(FM); 1, if $250 \geq \Delta K \leq 1 \times 10^6$ M^{-1}}



(b)

(Ca^{2+}, lectin)	2 (FL)	3 (FL)
(0,0)	0	0
(1,0)	0	0
(0,1)	0.35	0.28
(1,1)	1	1

Fig. 4 (a) Fuzzy logic of strong binding. (b) Truth table.



(Ca^{2+}, lectin)	Comp2 (FL)	Comp 3 (FL)
{0,0}	0	0
{1,0}	0; 0.11 (SM); 1.0 (TW)	0; 0.2 (SM); 1.0 (TW)
{0,1}	0; 0.35 (SS); 0.74 (FM)	0; 0.5 (SS); 0.64 (FM)
{1,1}	1 (S)	1 (S)

Fig. 5 FLS rules: $\Delta K \leq 250\ M^{-1}$ – weak interaction, $100 \leq \Delta K \leq 9 \times 10^6\ M^{-1}$ – medium interaction and for $\Delta K \geq 1 \times 10^6\ M^{-1}$ – strong binding. SM (slightly medium); TW (true weak); SS (slightly strong); FM (fairly medium); FW (fairly weak); (b) truth table.

finally, 'strong binding' (z) = {0–1, if $1 \times 10^6 \geq \Delta K \leq 9 \times 10^6\ M^{-1}$; 1, if $\Delta K \geq 9 \times 10^6\ M^{-1}$}. The FLS that characterizes specific CPIs of **2** is presented in Fig. 5a. The red vertical line represents the Ca^{2+} binding of **2** ({1, 0}) with binding affinity 115 M^{-1} and three degrees of interaction. The pointing of the red arrow to zero may be interpreted as 'no-binding' or low output (0) (as in BL). The black arrow (pointing to 0.11) may be described as 'slightly medium (SM) binding' and light blue arrow (pointing to 1.0) – 'true weak (TW) binding'. In general, Ca^{2+} ions mediated interactions can be considered as real values, ranging from 0 to 1, with three degrees of weak interactions. Similarly, PNA lectin interactions with **2** (green vertical line) again displayed three degrees of medium interaction. The red arrow indicates 'no-binding' and black arrow (pointing to 0.35) may be described as 'slightly strong (SS) binding'. The light blue arrow at 0.74 indicates 'fairly medium (FM) binding'. Similarly, the black line ({1, 1}) represents 'strong binding' or output 1. Similarly, the experiment with **3** showed three degrees of interactions.

In this communication, we have introduced the use of the two logic systems, FLS and BL, for presenting carbohydrate–protein interactions in glycoclusters composed of multivalent β-cyclodextrin appended with lactose molecules and peanut agglutinin lectin. We compare between the two logic systems, emphasize and demonstrate the preference of FLS over BL, which describes only the two states 1 and 0. FLS provides a real-time analytical tool for exploring these systems, and is adequate for presenting transitions from one state to the other, namely from 'non-binding' to 'binding' states. Degrees of interactions between the glycoclusters and PNA lectin, with and without calcium Ca^{2+} ions, were used as the linguistic variables for the fuzzy logic sets. The association constants, between these parameters, measured by ITC, were the output.

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