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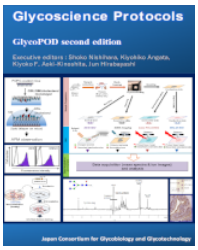

Binding assay of lectins and glycoproteins by surface plasmon resonance

Yuka Kobayashi, PhD[1]

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Introduction

Surface plasmon resonance (SPR) has been widely used for biomolecular interaction analysis. It can also be used to study lectin–sugar interactions and is characterized by the fact that the interactions can be analyzed without performing any labeling operations and they can be observed in real time. It is highly sensitive and efficient for label-free interaction analyses. In this protocol, we introduce the Biacore instrument as a research tool for investigating specific interactions of glycoconjugates (1-4). To measure the interaction of the analyte (lectins) with the ligand, the glycoproteins fetuin, thyroglobulin, and mucin (porcine stomach mucin: PSM) are immobilized on a sensor surface. In this model study, *Canavalia ensiformis* agglutinin (ConA), *Erythrina cristagalli* agglutinin (ECA), *Lens culinaris* agglutinin (LCA), *Triticum vulgaris* agglutinin (WGA), *Sambucus sieboldiana* agglutinin (SSA), and *Maackia amurensis* agglutinin (MAM) were used as analytes. Different concentrations of lectins are injected into the glycoprotein-immobilized flow cells, and the corresponding responses are recorded (the following concentrations of each lectin are used: 0, 62, 125, 250, and 500 nM). The obtained results are analyzed using biospecific interaction analysis (BIA) evaluation software. It includes a module for performing kinetic analysis, with the ability to overlay sensorgrams for presentations and/or calculation of kinetic parameters, such as k_a and k_d, and a concentration module that enables concentration measurements of specifically interacting analytes.

Protocol

This protocol describes essential steps of SPR analysis of lectins using glycoprotein-immobilized sensor chips comprising immobilization of glycoprotein ligands, binding analysis using plant lectins, and data evaluation.

Materials

1. Purified glycoproteins as ligands: fetuin, thyroglobulin, PSM (Sigma-Aldrich, Burlington, MA) (**Note 1**)
2. Purified lectins as analytes: ConA, ECA, LCA, WGA, SSA, MAM (Cosmo Bio Co., Ltd, Tokyo, Japan, JCM#J103, J105, J107, J120, J118, J110)
3. Sensor chip CM-5 (Cytiva, Tokyo, Japan, BR-1005-30)
4. Running buffer: HBS-EP (10 mM of HEPES, pH 7.4, 150 mM of NaCl, 3 mM of EDTA, 0.05% surfactant P20, and Cytiva: BR-1006-69).

Author Affiliation: 1 J-Chemical INC.; Email: yuka.kobayashi@j-oil.com.

Corresponding author.

5. Amine coupling kit (ethylene dichloride [EDC] [*N*-ethyl-*N*'-(3-dimethylaminopropyl) carbodiimide hydrochloride), NHS (*N*-hydroxysuccinimide), ethanolamine-HCl; Cytiva: BR-10000-50
6. 10 mM of sodium acetate, pH 4.0
7. Regeneration reagents for the binding assay: 0.2 M mannose, 0.2 M galactose, 0.2 M glucose, 0.2 M *N*-acetylglucosamine, or 0.2 M lactose dissolved in HBS-EP

Instruments

1. BiacoreT100 (Cytiva)
2. Evaluation Software (Cytiva)

Methods

1. Real-time detection of six different lectins binding to various glycoproteins (e.g., fetuin, thyroglobulin, porcine stomach mucin) can be performed using a BiacoreT100. Glycoproteins are covalently immobilized *via* their primary amines to carboxyl groups on the carboxymethyl (CM)-dextran layer of the sensor chip CM-5 using a protocol from the manufacturer's specifications.
2. Immobilization of ligand
    a. Set up the reference cell as follows: activate the reference sensor chip Fc1 with 100 µL of 0.4 M EDC and 100 µL of 10 mM of NHS and block with 150 µL of 1 M ethanolamine (pH 8.5) at 25°C (**Note 2**).
    b. Immobilize the glycoprotein as follows: activate sensor chips Fc2, 3, and 4 with 100 µL of 0.4 M EDC and 100 µL of 10 mM of NHS at 25°C.
    c. For chip Fc2, pass 100 µL of 200 µg/mL of fetuin in 10 mM of sodium acetate, pH 4.0, loaded at a rate of 5 µL/min.
    d. For chip Fc3, pass 100 µL of 200 µg/mL of thyroglobulin in 10 mM of sodium acetate, pH 4.0, at a rate of 5 µL/min.
    e. For chip Fc4, pass 100 µL of 200 µg/mL of porcine stomach mucin in 10 mM of sodium acetate, pH 4.0, at a rate of 5 µL/min.
    f. Cap chips Fc2, 3, and 4 by exposing them to 150 µL of 1 M ethanolamine (pH 8.5).
    g. Determine the amount of immobilization: determine the amount of immobilized ligand. Data points can be taken before initiating the first step and then at the end of the last step. The difference in signal intensity represents the amount of immobilized ligand in resonance units (RU); 1,000 RU is equivalent to a mass change of approximately 1 ng/mm^2 surface area. The final immobilization levels are 2076 RU (fetuin immobilized cell, Fc2), 2003 RU (immobilized thyroglobulin cell, Fc3), and 1209 RU (porcine stomach mucin immobilized cell, Fc4), respectively (shown in Figures 1, 2, and 3) (**Note 3**).
3. Binding analysis
    a. Prepare samples of six lectins in HBS-EP at concentrations of 31.3, 62.5, 125, 250, and 500 ng/mL.
    b. Before loading the analytes, equilibrate the chip with 10 mM of HEPES buffered saline (pH 7.4) containing 3 mM of EDTA and 0.005% of the surfactant P-20 (HBS-EP).
    c. Perform all analyses at a flow rate of 20 µL/min.
    d. Inject each analyte at various concentrations in the same buffer (samples) over the immobilized ligand.
    e. After injection of the analyte, load HBS-EP onto the sensor surface to initiate dissociation.
    f. Regeneration: Regenerate the chip by injecting 100 µL of 0.2 M haptenic sugars (mannose for ConA, galactose for ECA, glucose for LCA, *N*-acetylglucosamine for WGA, lactose for SSA and MAM) and wash with HBS-EP (**Note 4**).
4. Evaluation of data

a Kinetics: SPR instrument software (evaluation software) includes a module for the kinetic analysis with the ability to overlay sensorgrams for the presentation and calculation of kinetic parameters, such as association and dissociation rate constants (k_a and k_d), and a concentration module enabling concentration measurements of the specifically interacting analyte (**Note 5**). As an example, the analysis data are shown in Tables 1, 2, and 3, and Figures 4, 5, and 6.

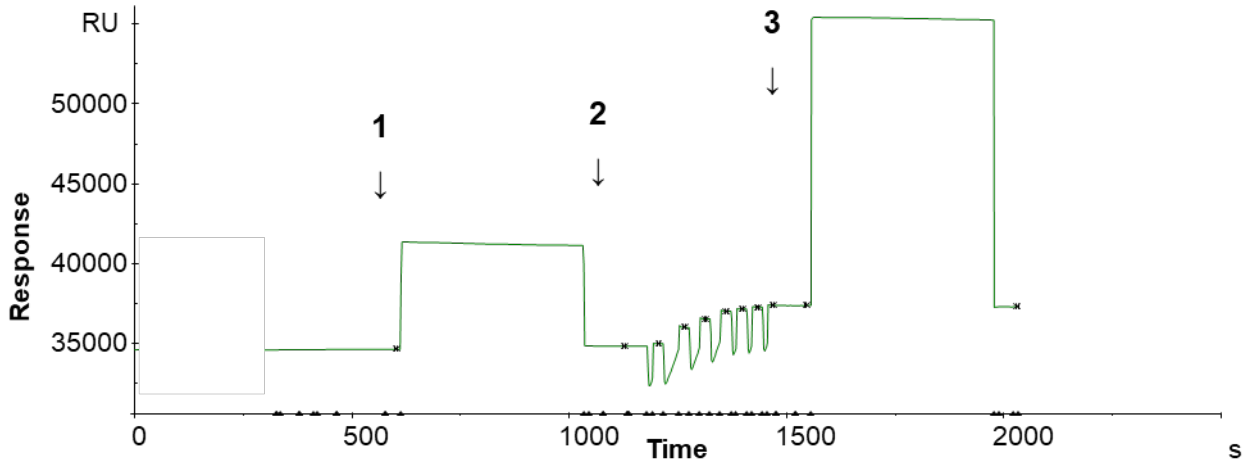

Figure 1: Sensorgram showing the immobilization of fetuin to the sensor surface.

Fetuin was immobilized to sensor chip CM5 via amine coupling. The arrows indicate the injection points of the solutions for each step. 1: The sensor was first activated with a mixture of ethylene dichloride and *N*-hydroxysuccinimide. 2: Fetuin dissolved in 10 mM of sodium acetate, pH 4.0, was then injected. 3: Thereafter, ethanolamine was injected to deactivate any remaining active esters.

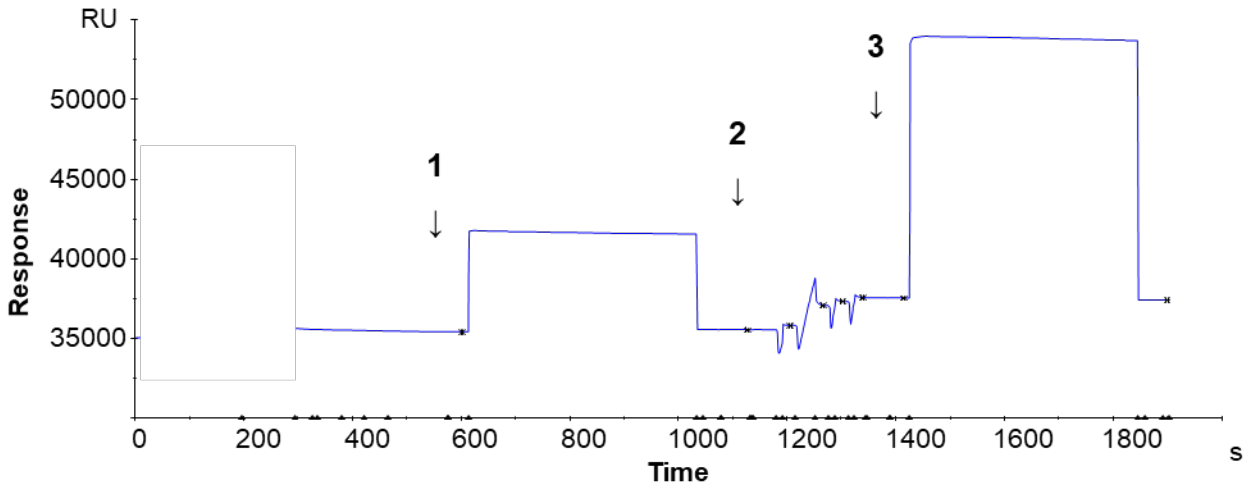

Figure 2: Sensorgram showing the interaction between immobilized thyroglobulin.

Thyroglobulin was immobilized to sensor chip CM5 via amine coupling. The arrows indicate the injection points of the solutions for each step. 1: The sensor was first activated with a mixture of ethylene dichloride and N-hydroxysuccinimide. 2: Thyroglobulin dissolved in 10 mM of sodium acetate, pH 4.0, was then injected. 3: Thereafter, ethanolamine was injected to deactivate any remaining active esters.

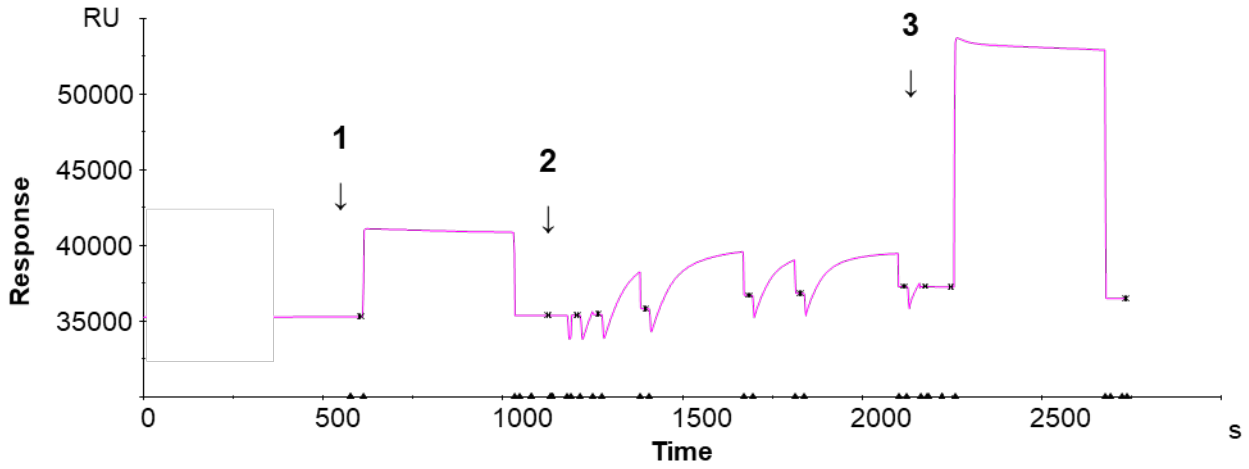

Figure 3: Sensorgram showing the interaction between immobilized porcine stomach mucin (PSM).

PSM was immobilized to sensor chip CM5 via amine coupling. The arrows indicate the injection points of the solutions for each step. 1: The sensor was first activated with a mixture of ethylene dichloride and *N*-hydroxysuccinimide. 2: Thyroglobulin dissolved in 10 mM of sodium acetate, pH 4.0, was then injected. 3: Thereafter, ethanolamine was injected to deactivate any remaining active esters.

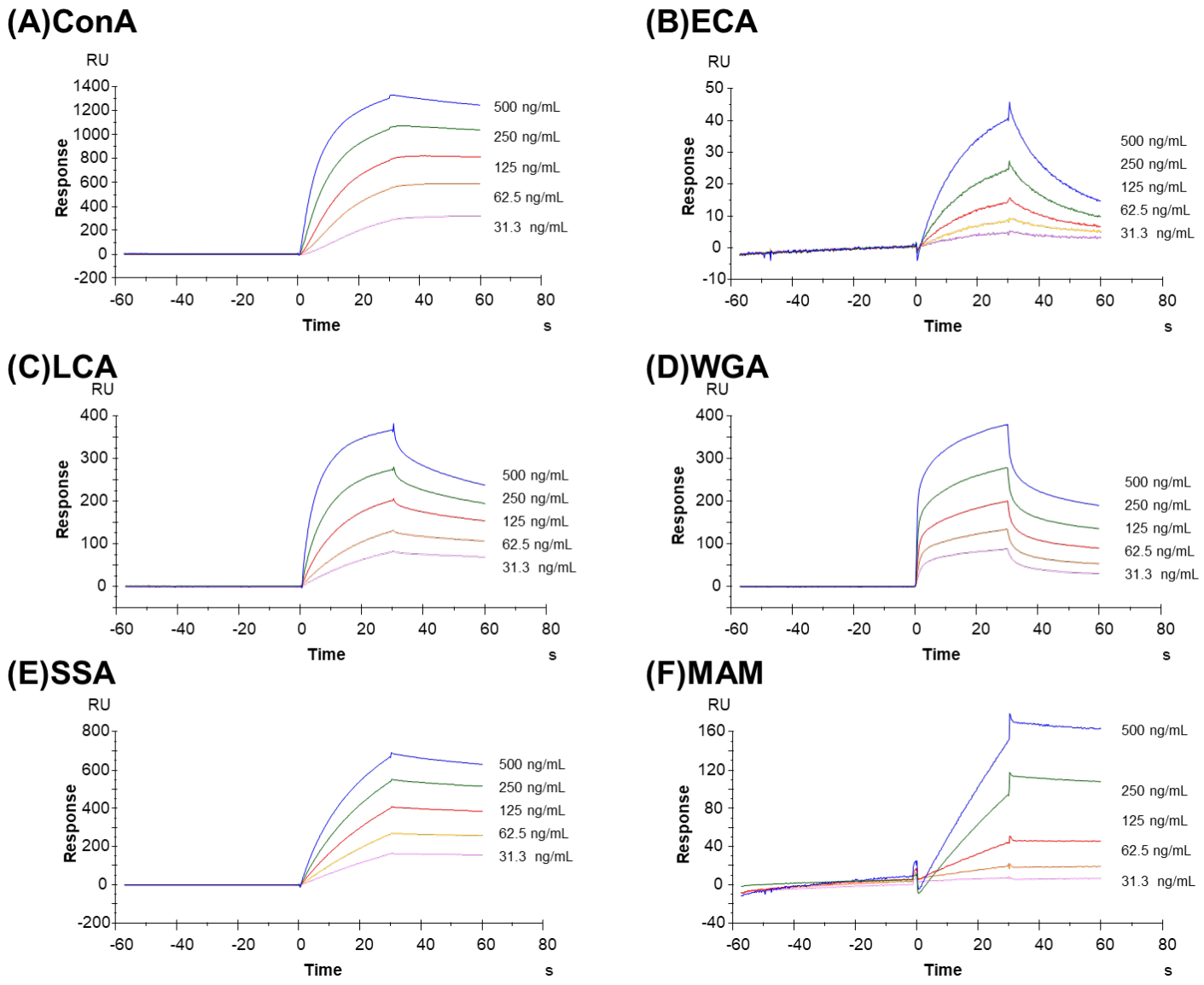

Figure 4: Sensorgrams showing the interaction between immobilized fetuin and lectins. A, Analyte, ConA; B, ECA; C, LCA; D, WGA; E, SSA; F, MAM (31.3、62.5, 125, 250, and 500 ng/mL).

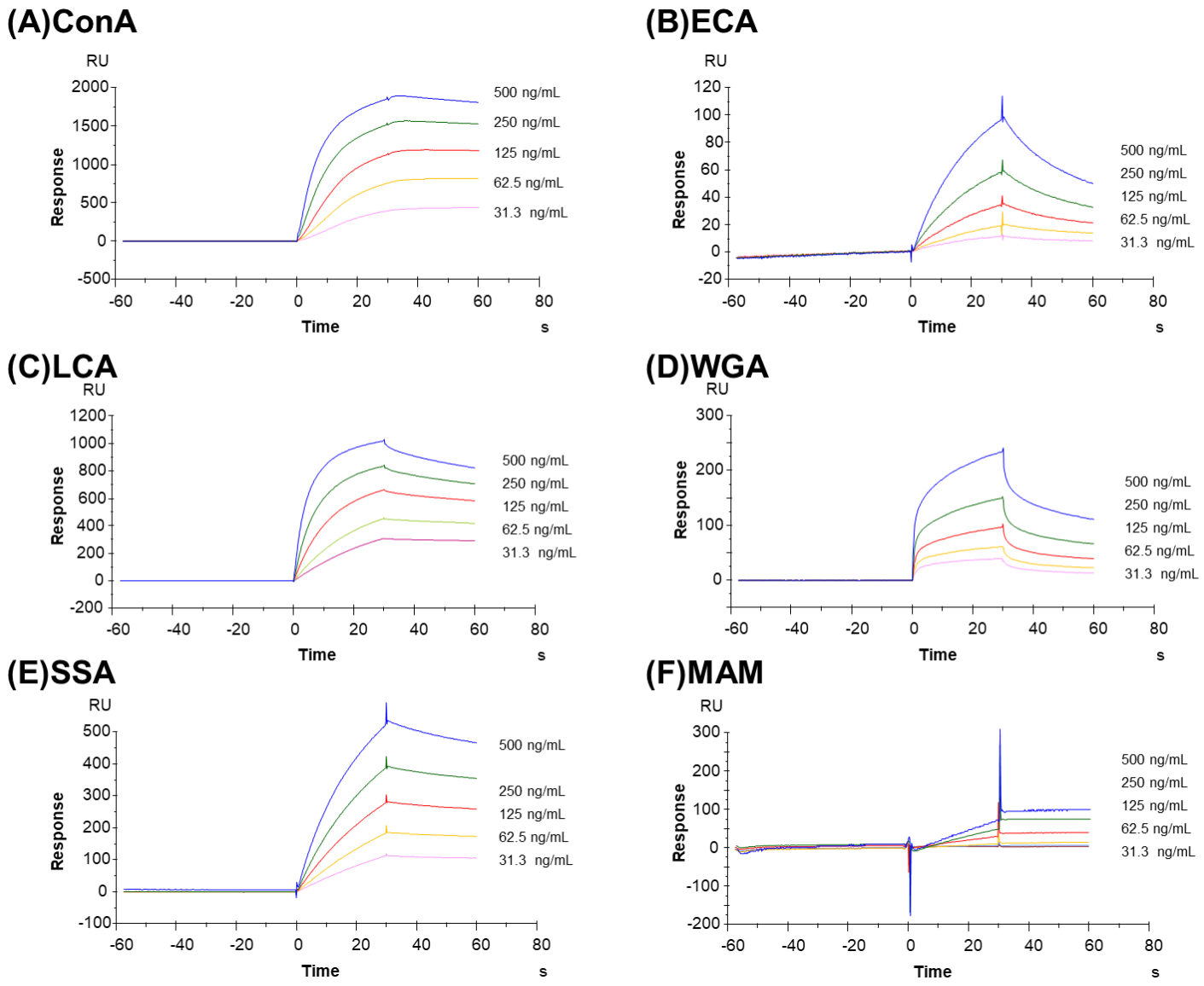

Figure 5: Sensorgrams showing the interaction between immobilized thyroglobulin and lectins. A, Analyte, ConA; B, ECA; C, LCA; D, WGA; E, SSA; F, MAM (31.3、62.5, 125, 250, and 500 ng/mL).

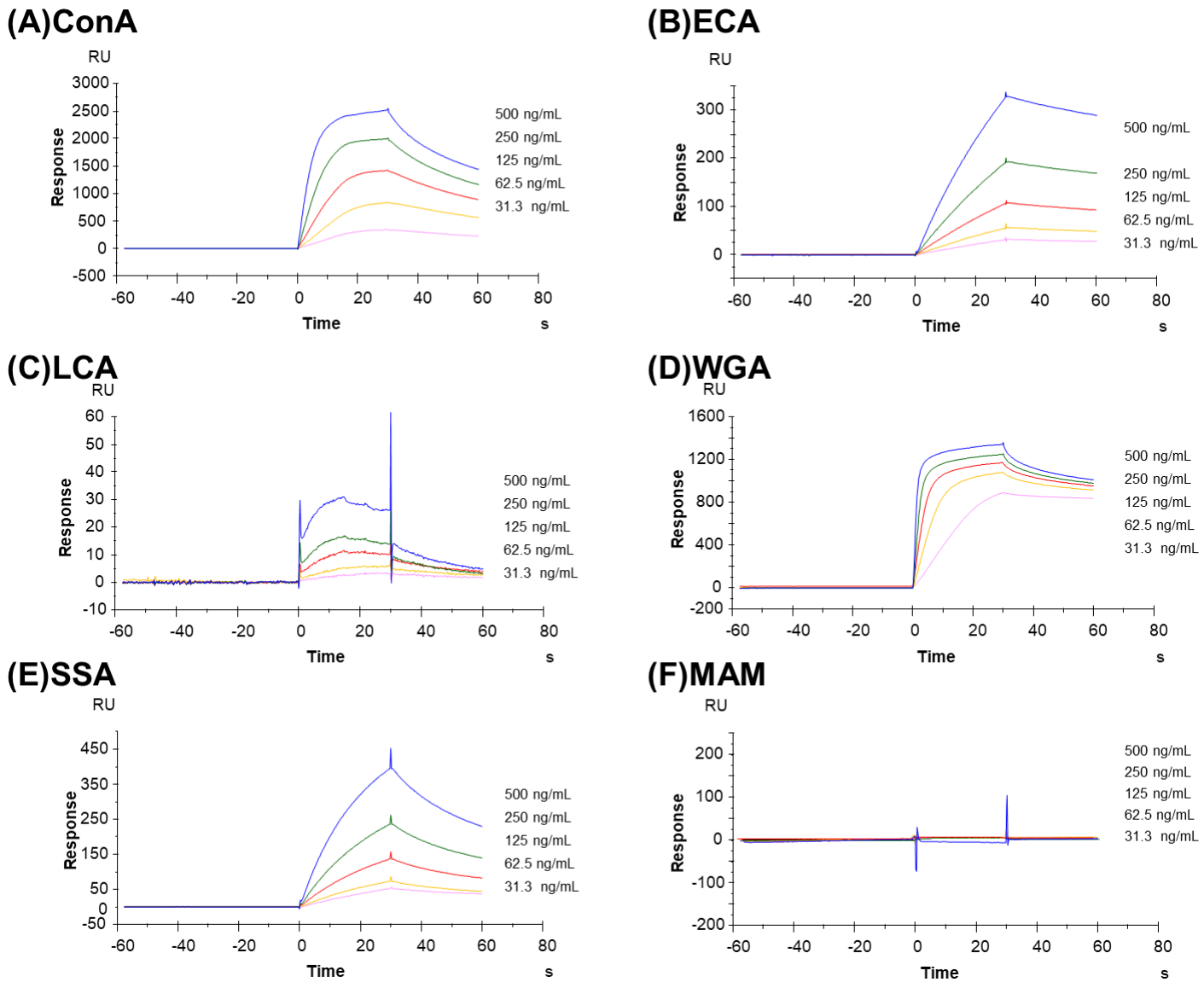

Figure 6: Sensorgrams showing the interaction between immobilized PSM and lectins. A, Analyte, ConA; B, ECA; C, LCA; D, WGA; E, SSA; F, MAM(31.3、62.5, 125, 250, and 500 ng/mL).

Table 1: Binding kinetics of the interaction between immobilized fetuin and lectins

Analyte	k_a ($M^{-1}S^{-1}$)	k_d (S^{-1})	K_D(M)
ConA	7.31×10^{3}	1.78×10^{-3}	2.43×10^{-8}
ECA	2.60×10^{3}	3.72×10^{-2}	2.43×10^{-6}
LCA	7.80×10^{4}	1.12×10^{-2}	1.44×10^{-7}
WGA	5.39×10^{4}	1.49×10^{-2}	2.77×10^{-7}
SSA	6.06×10^{4}	2.09×10^{-3}	3.45×10^{-8}
MAM	7.66×10^{2}	2.30×10^{-3}	3.00×10^{-6}

Table 2: Binding kinetics of the interaction between immobilized thyroglobulin and lectins

Analyte	k_a ($M^{-1}S^{-1}$)	k_d (S^{-1})	K_D(M)
ConA	7.02×10^4	8.99×10^{-4}	1.28×10^{-8}
ECA	2.37×10^4	2.21×10^{-2}	9.32×10^{-7}
LCA	9.81×10^4	5.11×10^{-3}	5.21×10^{-8}
WGA	2.58×10^4	1.56×10^{-2}	6.04×10^{-7}
SSA	8.46×10^4	2.88×10^{-3}	3.40×10^{-8}
MAM	3.49×10^2	5.28×10^{-3}	1.51×10^{-5}

Table 3: Binding kinetics of the interaction between immobilized porcine stomach mucin and lectins

Analyte	k_a ($M^{-1}S^{-1}$)	k_d (S^{-1})	K_D(M)
ConA	7.58×10^4	1.90×10^{-2}	2.51×10^{-7}
ECA	1.25×10^4	4.49×10^{-3}	3.58×10^{-7}
LCA	1.28×10^5	3.30×10^{-2}	2.57×10^{-7}
WGA	1.78×10^7	2.38×10^{-2}	1.33×10^{-9}
SSA	1.96×10^4	1.94×10^{-2}	9.91×10^{-7}
MAM	n. i. [a]	n. i.	n. i.

[a] n. i.; no interaction.

Notes

1. Immobilized ligand should have purity greater than 90%.
2. Confirmation of a specific interaction response requires a blank reference surface.
3. The amounts of immobilized ligands can be controlled according to the purpose of the study.
4. Owing to the availability of low-cost simple sugars (monosaccharides or disaccharides), it is convenient to use these carbohydrates to clean the sensor chips instead of using harsher cleaning solutions, such as acids or alkali. In this way, the immobilized glycoprotein is stable for multiple experiments.
5. To calculate the rate constants, the samples are appropriately diluted in HBS-EP at various concentrations. k_a and k_d are calculated using BIA evaluation software (Cytiva). The affinity constants (K_Ds) were calculated using k_a and k_d.

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