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Electronic Supplementary Information

Selective Adsorption of Carbohydrates and Glycoproteins via Molecularly Imprinted Hydrogels: Application to a Visible Detection by a Boronic Acid Monomer

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Materials and Instruments.

PEG 600 dimethacrylate (14G-DMA) was kindly donated from Shin-Nakamura Chemical (Wakayama, Japan) and utilized as received. 3-aminophenylboronic acid was purchased from Tokyo Chemical Industry (Tokyo, Japan), sodium hydroxide (NaOH), glucose, sodium hydrogen carbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3), phenol, mannitol, maltose, maltopentaose, ammonium persulphate (APS), and tetramethylethylenediamine (TEMED) were from Wako Pure Chemical Industries (Osaka, Japan), 4-vinylphenylboronic acid (VPB), ribonuclease B (RNase B), 4-aminostyrene, and fetuin were from Sigma-Aldrich Japan (Tokyo, Japan), and hydrochloric acid (HCl), mannose, fructose, phosphoric acid, sulfuric acid, sodium nitrite, methanol, ethyl acetate, and chloroform were from Nacalai Tesque (Kyoto, Japan), horseradish peroxidase (HRP) was from TOYOBO (Osaka, Japan), and ribonuclease A (RNase A) was from TAKARA BIO (Kusatsu, Japan). Deionized water was obtained from a Milli-Q Direct-Q 3UV system (Merck Millipore, Tokyo, Japan).

A UV-2450 (Shimadzu, Japan) as a UV-Vis spectrophotometer, JNM-ECA500 (JEOL, Japan) as a NMR spectrometer, Nicolet iS5 ATR (Thermo Fisher Scientific, USA) as an FT-IR spectrometer, and F-52 (HORIBA, Japan) as a pH meter were utilized for the evaluation of the synthesized compounds and prepared hydrogels.

Preparation of the hydrogels.

The molecularly imprinted hydrogel (MIH) and non-imprinted hydrogel (NIH) were prepared as follows: According to the previous study[7b], we confirmed the optimized composition of the hydrogel-preparation. The crosslinker, functional monomers, template, and radical initiator were mixed in a polypropylene tube, and then the homogeneous solution was degassed by N_2 gas bubbling. The solution was poured into a Pyrex tube (60 mm × 9.0 mm i.d.) and redox polymerization was carried out under ambient for 2 h. After polymerization, the hydrogel was cut into the 1.0 mm thickness. The templates and unreacted reagents were washed with 0.2 M aqueous phosphoric acid. To examine the effect of the functional monomers and templates containing carbohydrates/glycoprotein, variety of MIHs were prepared as shown in Tables S1 to S3.

Table 1. Compositions of the MIHs for carbohydrates.

	NIH-1	MIH (frc 2.5)	MIH (frc 5.0)	MIH (frc 10)	MIH (frc 20)	MIH (frc 50)	MIH (glu 10)
Crosslinker	14G-DMA, 228 μmol						
Monomer	VPB, 5.0 μmol						
Template	——	fructose, 2.5 μmol	fructose, 5.0 μmol	fructose 10.0 μmol	fructose, 20.0 μmol	fructose, 50.0 μmol	glucose 10.0 μmol
Initiator	10% APS solution, 5.70 μL; TEMED, 1.14 μL						
Solvent	10 mM carbonate buffer (pH 10.2), 3.0 mL						

Table 2. Compositions of the MIHs for RNase B.

	MIH (RB, 228)	MIH (RB, 257)	MIH (RB, 289)	MIH (RB, 314)	MIH (RB, 356)	MIH (RB, 428)
14G-DMA (μmol)	228	257	289	314	356	428
Monomer	VPB, 2.0 μmol					
Template	RNase B, 0.2 μmol					
Initiator	10% APS solution, 5.70 μL; TEMED, 1.14 μL					
Solvent	10 mM carbonate buffer (pH 10.2), 3.0 mL					

Table 3. Compositions of the MIHs for glycoproteins.

	NIH-2	MIH (HRP)	MIH (fetuin)	MIH (RN-B)
Crosslinker	14G-DMA, 228 μmol			
Monomer	VPB, 2.0 μmol			
Template	——	HRP, 0.2 μmol	fetuin, 0.2 μmol	RNase B, 0.2 μmol
Initiator	10% APS solution, 5.70 μL; TEMED, 1.14 μL			
Solvent	10 mM carbonate buffer (pH 10.2), 3.0 mL			

Batch adsorption of carbohydrates and glycoproteins.

The MIHs, in which the templates and unreacted reagents were completely removed by washing with aqueous phosphoric acid, were soaked into the carbohydrates or glycoproteins solutions for 24 h. Then, the amounts of adsorbed carbohydrates or glycoproteins were estimated by the determination of the free concentration in the supernatant with the UV-Vis spectrometer. For determination of carbohydrates, the supernatant of 0.2 mL was mixed with 5% phenol of 0.2 mL, and then sulfuric acid of 1.0 mL was added and the mixture was left at ambient for 10 min. After cooling in an ice bath, the concentration of carbohydrates was determined at UV 480 nm. For glycoproteins, the supernatant was directly measured at UV 280 nm. The analytical curves are shown in Fig. S1.

The adsorption amount, adsorption ratio, and imprinting factor were estimated by the following equations. All the evaluations were repeated with 3 pieces of each gel.

Adsorption ratio =
amount of adsorbed in experimental (mol) / amount of adsorbed in theoretical (mol) × 100 (%)

Imprinting factor = Adsorption ratio of MIH / Adsorption ratio of NIH

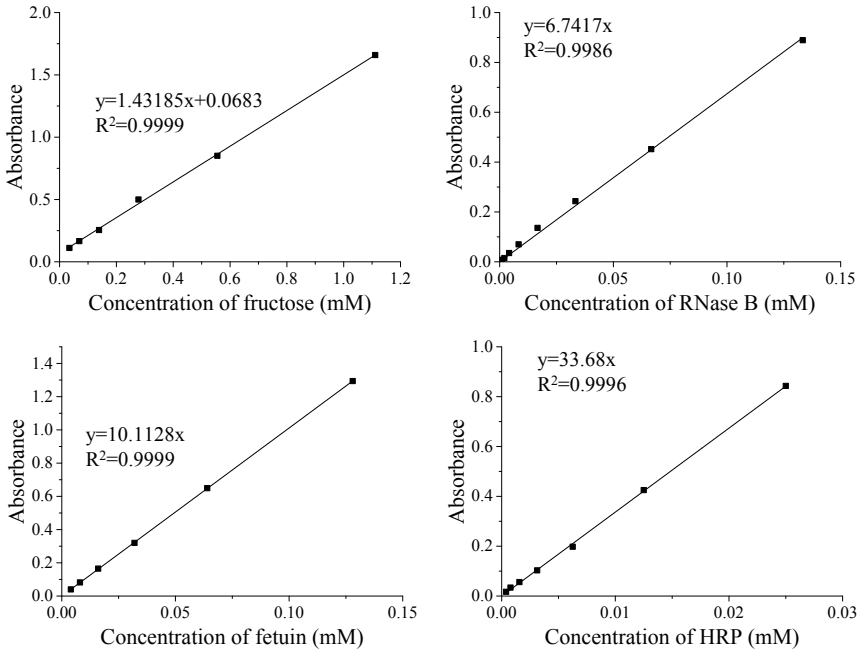
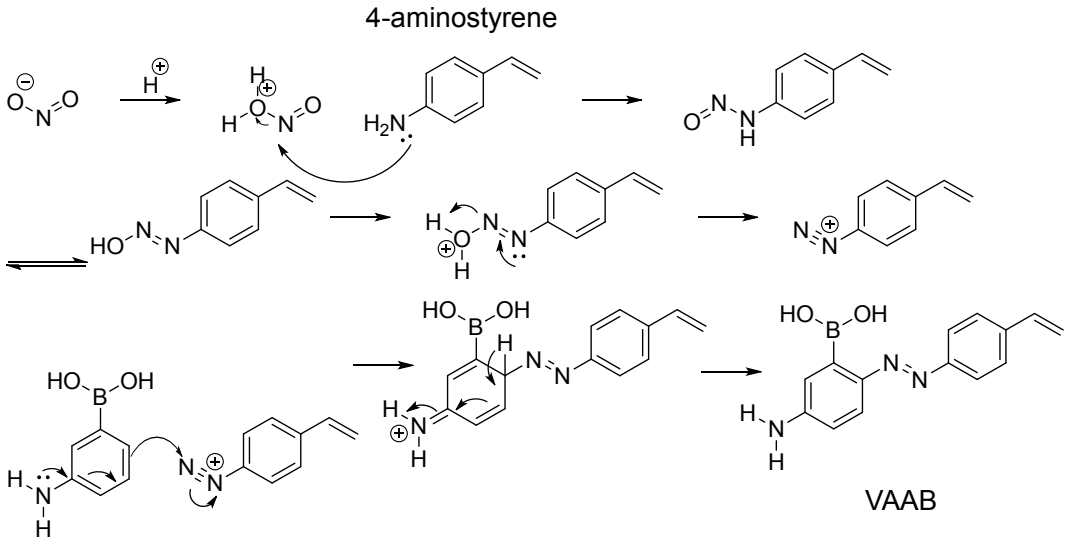


Fig. S1. Analytical curves by UV determination for fructose, RNase B, fetuin, and HRP.

Synthesis of 2-[(4-vinylphenyl)azo]-4-amino-benzeneboronic acid.

4-aminostyrene of 371.8 mg (3.0 mmol) was dissolved in 1.0 M HCl of 20 mL under 0 °C and $NaNO_2$ of 218.4 mg (3.0 mmol) in water of 10 mL was added. The mixture was stirred for 15 min. Then, 3-aminophenylboronic acid of 204.2 mg (1.5 mmol) in 1M NaOH of 10 mL was added to the solution. After neutralization by 1 M NaOH, the mixture was reacted at 0 °C for 2 H.

The products were extracted with ethyl acetate and dried with $MgSO_4$. After removal of the solvent, the residue was purified by two kinds of column chromatography. First, the residue was separated by silica gel chromatography with chloroform / methanol / acetic acid = 93 / 7 / 1 as a mobile phase and the fraction was dried with an evaporator. Then, the residue was separated by reversed-phase column chromatography with octadecyl-bonded silica as an adsorbent and water / methanol / formic acid = 20 / 80 / 0.5 as a mobile phase. Finally, the isolated compound was assigned by ^{1}H-NMR including COSY. The synthesis scheme was shown in Scheme S1.



Scheme S1. Synthesis of 2-[(4-vinylphenyl)azo]-4-amino-benzeneboronic acid

^{1}H-NMR (500 MHz, D_2O): δ/ppm 5.38, 5.93 (dd, 2H, (a,b)), 6.73 (d, 1H, (f)), 6.82 (s, 1H, (h)), 6.79-6.86 (m, 1H, (c)), 7.62 (d, 2H, (d)), 7.78 (d, 1H, (g)), 7.97 (d, 2H, (e)).

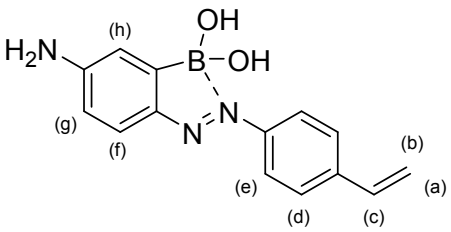


Fig. S2. Assignment of VAAB by ^{1}H-NMR.

Alteration of the absorption spectra of spectra on VAAB with fructose.

To examine the alteration spectrum, 1.0 mM VAAB in 10 mM carbonate buffer (pH 10.2) was mixed with variety of concertation of fructose, briefly 2.1 to 33.6 mM. Each solution was evaluated by a UV-Vis spectrometer.

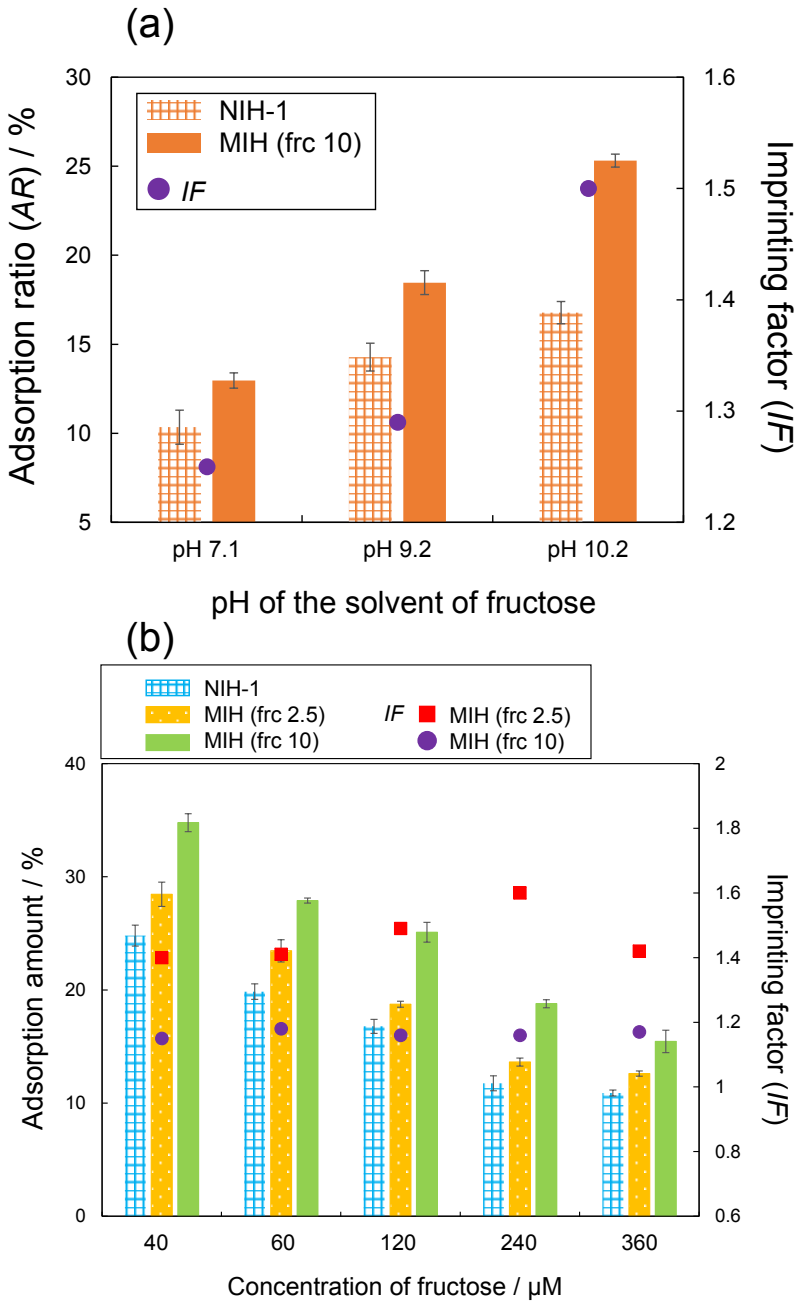


Fig. S3. Adsorption selectivity and imprinting factor for MIH (frc). (a) adsorption ratio of MIH (frc 10) against pH of the solutions. (b) adsorption amount and imprinting factor with MIH (frc) by changing the concentration of fructose for batch adsorption. ("adsorption amount" means the ratio of amount of fructose in the sample solution, which is different from the meaning of "adsorption ratio" in main text and ESI.

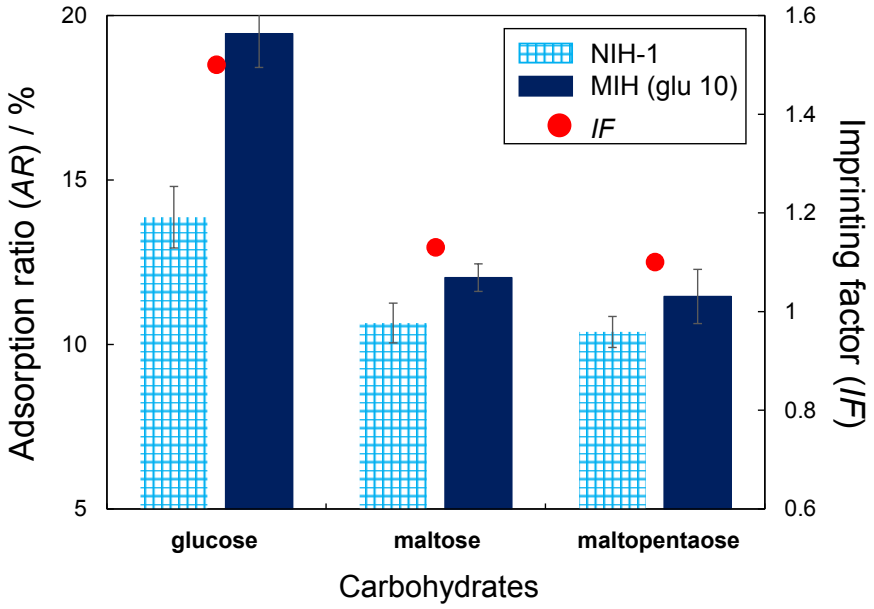


Fig. S4. Adsorption ratio and imprinting factor with MIH (glu) for several carbohydrates (240 μM in each).

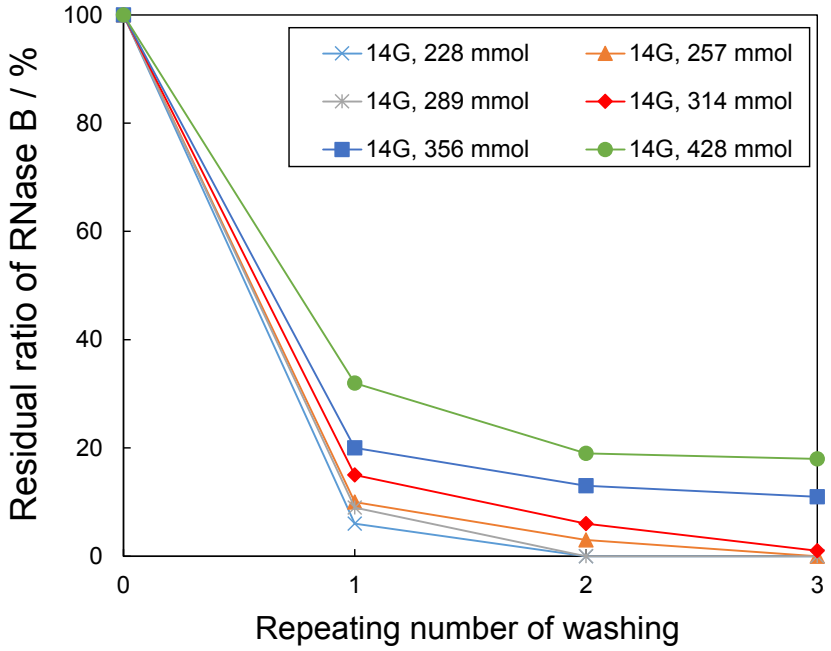


Fig. S5. Residual template molecules, RNase B by repeating washing. The MIHs prepared with several amount of crosslinker were washed with 0.1 M aqueous phosphoric acid and the un-removal RNase B was estimated.

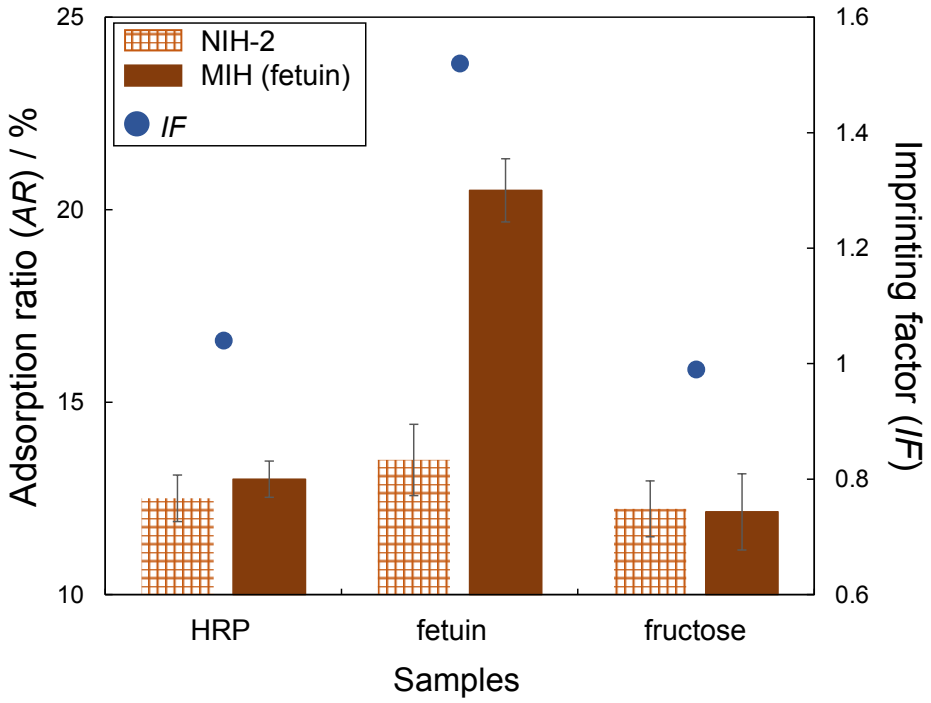


Fig. S6. Adsorption selectivity in MIH (fetuin). Each solution of HRP, fetuin (4.2 μM), and fructose (240 μM) was utilized for the batch adsorption.

Table S4. Characteristics of glycoproteins.

	Molecular weight	Number of diols	isoelectric point
HRP	40,000	63-90	8.8
fetuin	48,000	42-63	3.3
RNase B	15,000	6-10	9.4

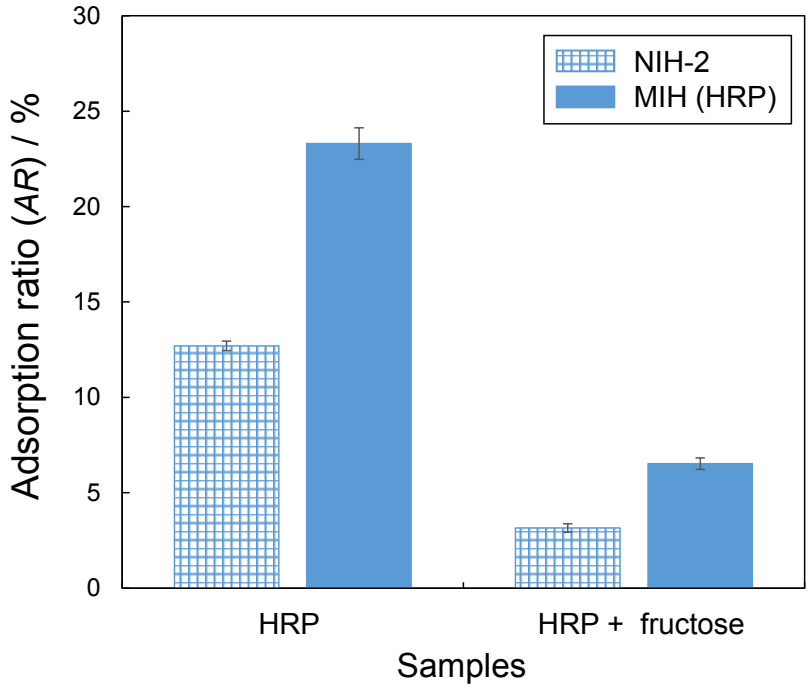


Fig. S7. Adsorption selectivity in MIH (HRP). In the mixture of HPR and fructose, the concertation of HRP and fructose were 4.2 μM and 240 μM, respectively.

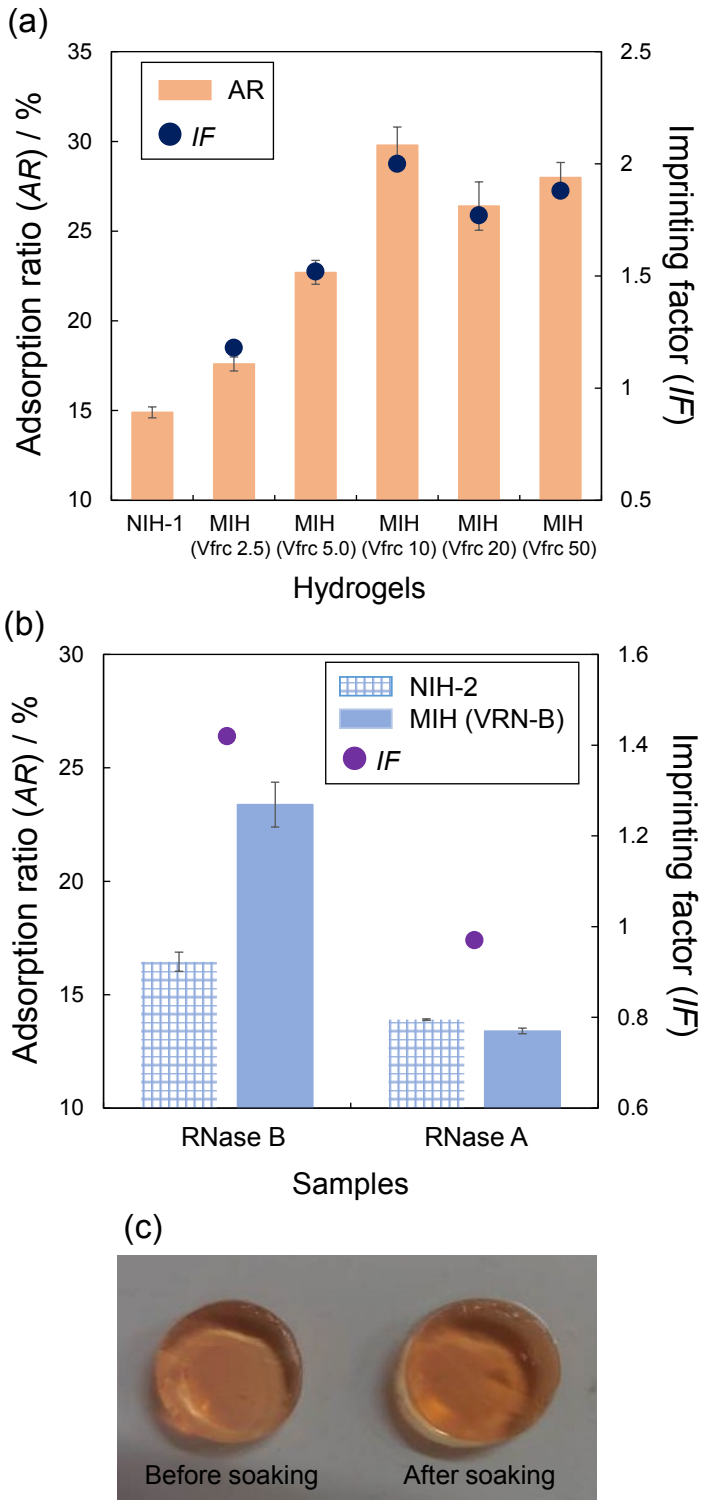


Fig. S8. Adsorption selectivity in MIHs with VAAB for fructose and RNase B. The MIHs were prepared with same manner as VPB-MIHs. The adsorption results for (a) MIH (Vfrc) and (b) MIH (VRN-B), (c) physical appearances of MIH (VRN-B) before and after soaking in RNase B solution.

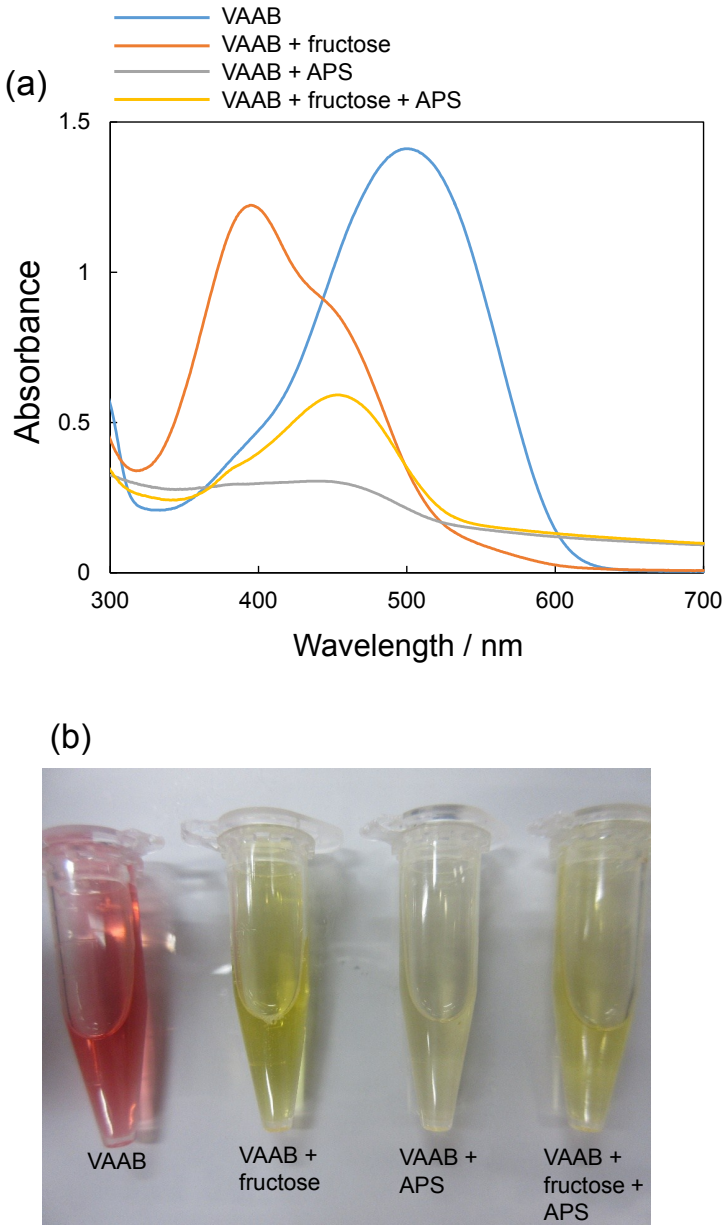


Fig. S9. Absorption spectra and physical appearances of VAAB solutions. (a) absorption spectra and (b) photos of VAAB with fructose and APS. All the same concertation (1 mM) were employed for the evaluation.