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# Influence of salt ions on binding to molecularly imprinted polymers

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**Abstract** Salt ions were found to influence on template binding to two model molecularly imprinted polymers (MIPs), targeted to penicillin G and propranolol, respectively, in water–acetonitrile mixtures. Water was detrimental to rebinding of penicillin G whereas propranolol bound in the entire water–acetonitrile range tested. In 100% aqueous solution, 3-M salt solutions augmented the binding of both templates. The effects followed the Hofmeister series with kosmotropic ions promoting the largest increase. Binding was mainly of non-specific nature under these conditions. In acetonitrile containing low amounts of water, the specific binding to the MIPs increased at addition of salts. Binding of penicillin G followed the Hofmeister series while an ion exchange mechanism was observed for propranolol. The results suggest that hydration of kosmotropic ions reduces the water activity in water-poor media providing a stabilizing effect on water-sensitive MIP–template interactions. The effects were utilized to develop a procedure for molecularly imprinted solid-phase extraction (MISPE) of penicillin G from milk with a recovery of 87%.

**Keywords** *Hofmeister effect; molecular imprinting; molecular recognition; penicillin G; propranolol; solid-phase*

## INTRODUCTION

Molecular imprinting is a method that provides synthetic polymers capable of predetermined selective recognition [1, 2]. Selectivity of the polymers originates from template molecules that interact with the polymer building blocks (i.e., monomers and cross-linkers) via covalent or non-covalent interactions. The templates direct the three-dimensional assembly of the building blocks during the polymerization. After extraction of the template from the molecularly imprinted polymer (MIP), recognition sites that are complementary to the template remain in the polymer network. The sites will rebind the template when the MIP is re-exposed to the template under conditions favoring binding. The recognition is believed to be a combination of shape selectivity and interactions between complementary functionalities of the template and the polymer. In addition to the specific binding that takes place in the recognition sites, the observed binding of a MIP can also contain a contribution from random non-specific interactions with the polymer. To estimate the degree of non-specific binding, control polymers (CPs) prepared either in the absence of

template or with a non-related template are normally evaluated in parallel.

The synthesis of MIPs is a complex process involving a large number of parameters whose interplay has not been fully elucidated [3]. Polymers were mainly developed by a trial-and-error approach and optimizations were often limited to variations of one parameter at a time in the beginning of the molecular imprinting era. More recently, several promising methods to approach the optimization problem in a more systematic way have been demonstrated. Chemometrical approaches, combinatorial synthesis, high-throughput screening, and computational methods have been applied to investigate factors of importance in the synthesis protocol and to expedite the optimization of polymer compositions towards high selective rebinding of the template [4–15].

The functional monomers are crucial since much of the selectivity of a MIP arises from template-monomer interactions. The monomers are typically chosen so that their functionalities complement the functionalities of the templates. The role of the cross-linker is to spatially lock the position of the functional monomers relative to the template and to provide

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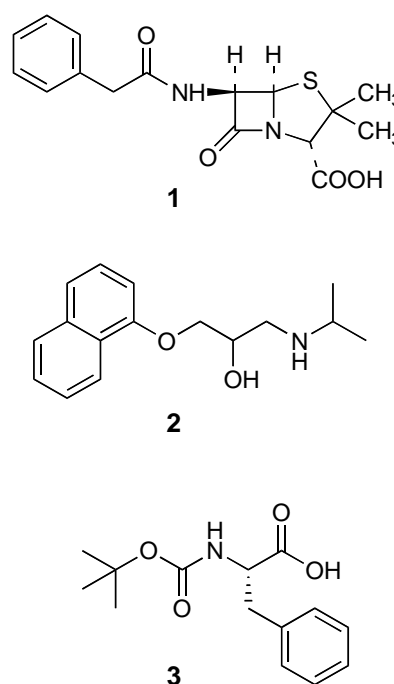
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rigidity to the polymer in order to form durable recognition sites. The porogenic solvent used during the polymerization and the rebinding solvent are other factors that affect the recognition and the binding characteristics. Water may interfere with hydrogen bonds involved in the recognition and MIPs relying on non-covalent interactions to the template are therefore often prepared in aprotic organic media. Optimal rebinding is usually observed in similar aprotic organic solvents as the ones used during the imprinting, although several cases of successful rebinding in aqueous media have been reported [8, 16–25]. The pH and the ionic strength of the rebinding solvent mixture have been recognized as parameters affecting the binding capacity [16, 19, 26]. The influence of the type of the salt has, however, so far been neglected.

For more than a century it has been known that salt ions affect a diverse range of chemical and biological phenomena and that different ions do so with different effectiveness. This ion effect has been named the Hofmeister effect after Franz Hofmeister, who studied the influence of ions on protein precipitation [27, 28]. The Hofmeister series ranks ions after their ability to promote an observable response. Hofmeister ascribed salts a “water absorbing effect.” Although there has been a debate on the mechanism behind the Hofmeister effect, many of the theories support the idea that ions have an impact on the hydrogen bonding of water [29]. Kosmotropic ions have a higher charge density than chaotropes and are more hydrated than the latter. We hypothesized that the Hofmeister effect might affect the binding of MIPs in aqueous rebinding media and that addition of kosmotropic ions would favor hydrogen bonding between the template and the MIP by decreasing interference from water molecules in the recognition sites. The hypothesis was tested on two poly(methacrylic acid-co-trimethylolpropane trimethacrylate) MIPs imprinted with penicillin G and propranolol (structures **1** and **2** in Scheme 1), respectively. Binding to the former MIP relies on hydrogen bonds [6] while the template recognition in the latter case relies on an ion exchange mechanism [16]. The present study highlights the influence of the Hofmeister effect on the two MIP model systems and furthermore demonstrates how the ion effect can be used practically to manipulate the binding to a MIP. A procedure for solid-phase extraction (SPE) of penicillin G from milk was developed. The interest in this antibacterial drug, commonly used in veterinary medicine for therapeutic and prophylactic treatment of infections, arises from the need of novel efficient detection methods. A serious problem associated with extensive use and spread of antibiotics is the development of antibiotic resistant bacterial strains [30]. Allergic reactions in hypersensitive milk consumers and inhibition of starter cultures in the dairy industry are additional problems arising from the use of antibiotics in livestock [31]. These concerns have resulted in legislative measures and establishment of maximum residue levels (MRLs) of antibiotics in foodstuffs [32].



**Scheme 1.** Structure of Penicillin G (**1**), Propranolol (**2**), and Boc-L-Phe-OH (**3**).

## EXPERIMENTALS

### Reagents

Penicillin G sodium salt and penicillin G potassium salt were purchased from Sigma (St. Louis, MO). (*RS*)-Propranolol hydrochloride, methacrylic acid (MAA), trimethylolpropane trimethacrylate (TRIM), and mineral oil (white heavy) were obtained from Aldrich (Milwaukee, WI). 2,2'-azobis(2-methylpropionitrile) (AIBN) was purchased from Acros (Geel, Belgium). Boc-L-Phe-OH was from Advanced ChemTech (Louisville, KY). (*RS*)-[4-<sup>3</sup>H]propranolol hydrochloride and [phenyl-4(*n*)-<sup>3</sup>H]benzylpenicillin were obtained from GE Healthcare (Buckinghamshire, UK). Organic solvents (p.a. grade) were purchased from Merck (Darmstadt, Germany). Salt and buffer solutions were prepared by dissolving the salts in purified and deionized water (Milli-Q). Scintillation liquid Ecoscint XR was purchased from National Diagnostics (Hessle Hull, England). The free amine of propranolol was prepared as described by Schweitz et al [33].

### Synthesis of spherical MIP and CP beads

MIP and CP pre-polymerization solutions were prepared as previously described by dissolving template, MAA, TRIM, and AIBN in acetonitrile according to the amounts given in Table 1 [6, 9, 26]. The pre-polymerization solutions were purged with a stream of nitrogen gas for 5 min. Spherical beads were synthesized by dispersing 25 mL of the pre-polymerization solutions in 475 mL of mineral oil with a model D125 basic dispersing device equipped with

an S25N-25F dispersing tool (IKA-werke GmbH & Co., Staufen, Germany) at 13 500 rpm for 1.5 min (MIP1 and CP1) or 3 min (MIP2 and CP2). The droplets formed were polymerized for 30 min at 315–395 nm under a Dymax UV curing flood lamp model PC-2000 (Torrington, CT) placed in a Sanyo MIR-553 cooled incubator set at 20 °C.

### Fractionation and microwave-assisted extraction of MIPs and CPs

The beads were wet-sieved in methanol (MeOH) with sieves from Retsch (Haan, Germany). Two major fractions were obtained; <25 µm and 25–50 µm. The smaller sized fraction (<25 µm) was allowed to sediment in acetone and the fine non-sedimented beads were removed. The remaining beads of this fraction were subjected to microwave-assisted extraction with MeOH–HOAc (1:1), MeOH, and acetonitrile using a MARS 5 microwave accelerated reaction system (CEM Corp., Matthews, NC) equipped with HP-500 Plus vessels. Approximately 0.5 g of beads and 40 mL of extraction solvent were added to each vessel. The samples were subjected to 300 W microwaves with a ramp over 10 min up to 120 °C and then for 15 min at constant temperature (120 °C). The beads were extracted three times with each extraction solvent. Between each run, the beads were collected in SPE columns, washed with MeOH, and dried. The beads were finally dried in vacuum overnight.

### One-point binding studies

Binding capacities were estimated by 18-h incubations of 1-mL samples containing beads of MIPs or CPs (0.1 mg) and 1 nM of radioactive ligand (either [phenyl-4(*n*)-<sup>3</sup>H]benzylpenicillin or (*RS*)-[4-<sup>3</sup>H]propranolol hydrochloride) in the appropriate incubation solvent mixture in micro-centrifuge tubes on a shaking table. After centrifugation (30 min, 13 000 rpm), 0.5 mL of the supernatants were withdrawn and added to 3.5 mL of Exoscint XR scintillation liquid. Radioactivity was measured by liquid scintillation counting using a Beckman LS 6000TA and the bound amounts of penicillin G or propranolol, respectively, were calculated.

### Binding isotherms

Binding curves were obtained by incubating increasing concentrations (0–500 nM for 1% aqueous solutions and 0–5000 nM for 100% aqueous solutions) of either non-radioactive penicillin G salt or non-radioactive propranolol with radioactive ligands (1 nM) and MIP or CP (0.1 mg/mL). Samples containing penicillin G in 100% aqueous media were incubated for 5 h; all other samples were incubated for 18 h. Samples were processed as described in the one-point binding studies above. The free concentration of the radioligands was determined by liquid scintillation and the bound amounts were calculated. Binding data were fitted to one-site Langmuir isotherms using GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

### Molecularly imprinted solid-phase extraction (MISPE) of penicillin G in milk

Non-homogenized milk (0.7% (w/w) fat; Åsens lantmjölk, Skånemejerier, Sweden) was spiked with [phenyl-4(*n*)-<sup>3</sup>H]benzylpenicillin to a concentration of 10 nM. 2 mL of the spiked milk was diluted with 18 mL of 200 mM sodium citrate buffer pH 5 containing 4 M NaCl. The diluted sample was centrifuged twice (30 min, 9 500 rpm) and precipitated components were separated from the soluble milk sample by decantation. MIP1 and CP1 beads (50 mg) were packed into empty 1-mL ISOLUTE SPE column reservoirs fitted with 10 µm-frits at the bottom (Biotage AB, Uppsala, Sweden). SPE experiments were run in triplicate. Elutions were gravity-driven. Each column was equilibrated with 1 mL of 200 mM sodium citrate buffer pH 5 containing 4 M NaCl. 1 mL of the centrifuged soluble milk sample was applied to each column (loading). Each column was washed with 1 mL of 200 mM sodium citrate buffer pH 5 containing 4 M NaCl (wash 1) and then dried in vacuum overnight. Each column was then washed with 1 mL of acetonitrile containing 1% of 25 mM sodium citrate buffer pH 5 (wash 2). Elution was carried out with MeOH (3 × 1 mL; elution 1–3). 0.5 mL of each fraction collected was added to 3.5 mL of Exoscint XR scintillation liquid and the amount of radioligand was measured by liquid scintillation.

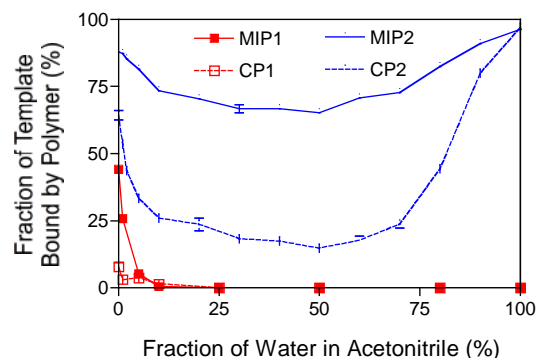
**Table 1.** Composition of pre-polymerization solutions.

Poly- mer	Template	Amount of Template		Amount of MAA		Amount of TRIM		Amount of AIBN		Amount of CH <sub>3</sub> CN
		(mmol)	(g)	(mmol)	(g)	(mmol)	(g)	(mmol)	(g)	
MIP1	Penicillin G (1)	1.94	0.692	19.4	1.670	29.1	9.849	0.78	0.127	14.55
CP1	Boc-Phe-OH (3)	1.94	0.515	19.4	1.670	29.1	9.849	0.78	0.127	14.55
MIP2	Propranolol (2)	6.0	1.558	18.0	1.551	18.0	6.098	2.4	0.395	18.0
CP2	Boc-Phe-OH (3)	6.0	1.592	18.0	1.551	18.0	6.098	2.4	0.395	18.0

## RESULTS AND DISCUSSION

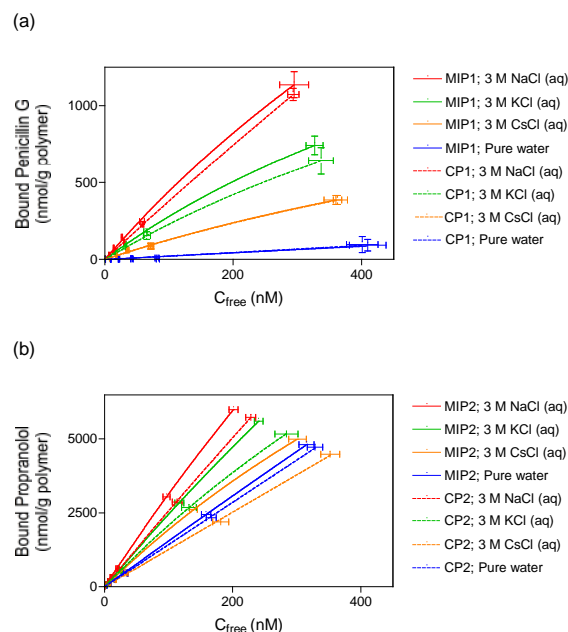
MIP microbeads targeted to either penicillin G (structure **1**, Scheme 1) or propranolol (structure **2**), were synthesized as model MIP systems by previously reported methods [6, 9, 26]. Droplets were formed by suspending the pre-polymerization solutions (Table 1), containing template, MAA, TRIM, and initiator, in mineral oil. The droplets were polymerized by photo-induced free-radical polymerization and the templates were subsequently removed from the polymer beads by microwave-assisted extraction. To estimate the degree of non-specific binding to the MIPs, corresponding CP beads were synthesized in parallel by substituting Boc-L-Phe-OH (structure **3**) for penicillin G and propranolol, respectively. The rationale for applying CPs prepared with a non-related template instead of non-imprinted polymers was to avoid overestimation of the imprinting effect. Non-imprinted polymers based on MAA as the functional monomer have shown to have lower surface areas than polymers prepared in the presence of templates [34–38]. Consequently, when non-imprinted polymers are applied as CPs, there is a potential risk that the non-specific adsorption is underestimated and the specific binding to the MIP is overestimated. Characterization of the surface area and porosity of MIPs and CPs are detailed in Supplementary Material (Table S1 and Fig. S1–S3).

The two MIP model systems were expected to behave differently with regard to their binding performance in the presence of water. Penicillin G imprinted poly(MAA-co-TRIM) MIPs have previously shown satisfactory binding in pure acetonitrile and acetonitrile containing small amounts of water [6]. Propranolol imprinted MIPs, on the other hand, show a substantially higher tolerance to water and were in fact among the first MIPs reported to work in aqueous media [16]. The influence of water on the binding of the templates to their respective MIPs, as measured by radioligand binding assays in acetonitrile–water mixtures, is demonstrated in Fig. 1.



**Fig. 1** Binding of  $^3\text{H}$ -labeled penicillin G (1 nM) to MIP1 (red filled squares, red solid line) and CP1 (red open squares, red dotted line) (0.1 mg/mL) and  $^3\text{H}$ -labeled propranolol to MIP2 (blue filled circles, blue solid line) and CP2 (blue open circles, blue dotted line) in water–acetonitrile mixtures. MIP1 was imprinted with the sodium salt of penicillin G and MIP2 with propranolol. Data points are the mean values of five replicates (MIP1 and CP1) and three replicates

(MIP2 and CP2), respectively. Standard errors are indicated with error bars.



**Fig. 2** Binding isotherms of (a) penicillin G to MIP1 (imprinted with the potassium salt of penicillin G) and CP1 and (b) propranolol to MIP2 and CP2. MIP binding is indicated with filled circles and solid lines; CP binding is indicated with open circles and dotted lines. Binding was carried out in 3-M solutions of NaCl (red), KCl (green), CsCl (orange), or pure water (blue). Data points are the mean values of five replicates and standard errors are indicated with error bars. It was ascertained that the observed decrease in concentration of penicillin G in the supernatant at the addition of salt was due to increased binding to the polymers and not to precipitation of penicillin G by carrying out corresponding control experiments without polymer present. The supernatants of these control samples showed no decrease in concentration when treated identically.

The amount of bound radioligand was calculated from the free concentration of ligand in the supernatant as measured by liquid scintillation. As expected, the two model systems behaved differently; binding of penicillin G to MIP1 was affected negatively by the addition of water and the binding profile approached zero already at 10% of water whereas MIP2 was able to bind propranolol in the entire water–acetonitrile mixture range. The highest binding of penicillin G to MIP1 was obtained in pure acetonitrile. In this rebinding solvent, and with a few percent of water added, the observed binding of penicillin G was substantially higher to the MIP than to the CP, indicating that the binding was mainly specific and due to an imprinting effect. With increasing amounts of water, both MIP1 and CP1 lost the ability to bind penicillin G, indicating that hydrogen bonds and polar interactions are crucial. In contrast, the binding of propranolol to MIP2 in pure acetonitrile was to a large extent of non-specific nature since significant binding was obtained to CP2 in this solvent. Furthermore, Fig. 1 shows that the binding to MIP2 decreased only slightly with increasing amount of water up to 50%. The binding to CP2 decreased even more in this interval resulting in a net increase in specific binding to the MIP. Above 50% of water, a rise in the binding profiles of propranolol to MIP2 and CP2 with increasing amount of water was observed. The rise was

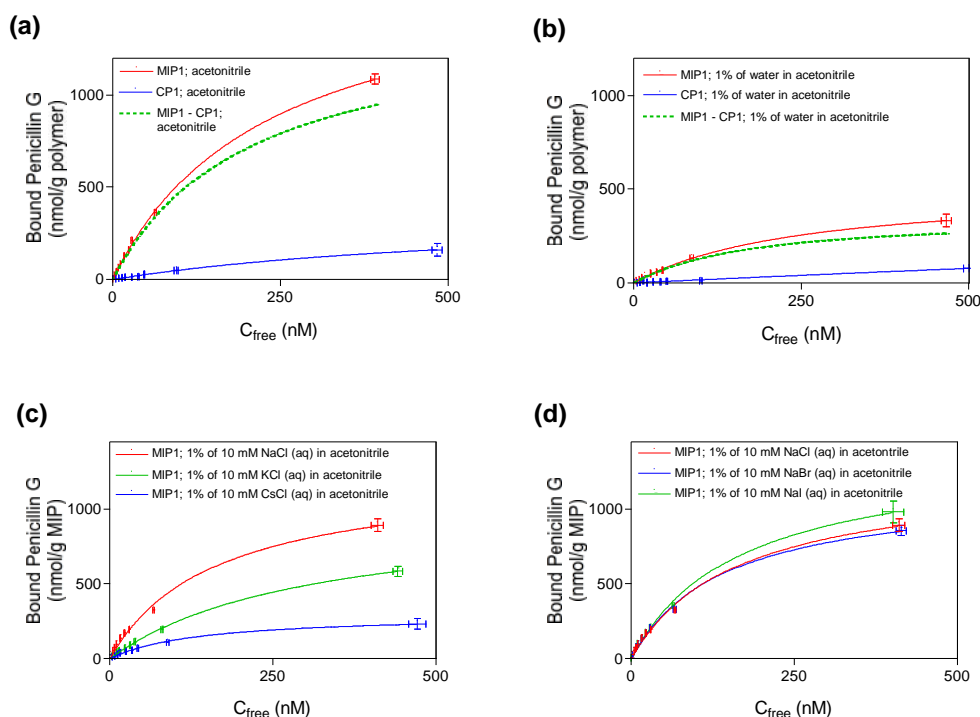
steeper for the binding to the CP than to the MIP, resulting in a net decrease in specific binding. In pure water, the propranolol binding appeared to be mainly of non-specific nature since similar high binding was obtained on both MIP2 and CP2.

Binding isotherms obtained by incubation of MIP1 and MIP2 with increasing concentrations of radiolabeled penicillin G and propranolol, respectively, in pure water confirmed the results obtained above; binding of penicillin G to both MIP1 and CP1 was negligible (Fig. 2a) whereas the binding of propranolol to MIP2 and CP2 was significant (Fig. 2b). The Hofmeister effect is observed typically in 100% aqueous media containing high salt concentrations. To test our hypothesis that binding can be improved by the addition of kosmotropic salt ions, binding isotherms were determined in 3-M solutions of NaCl, KCl, and CsCl (Fig. 2). The binding data show that the addition of salt had a dramatic effect on the binding to both MIPs and CPs. The salts augmented the binding in the order NaCl > KCl > CsCl. The order follows the Hofmeister series of cations, with sodium being the most kosmotropic cation. Although the binding to the MIPs was improved significantly by the addition of kosmotropic salt ions, the increased binding appeared to be mainly of non-specific nature since similar increases were obtained on the CPs. The same trend was seen also at higher template concentrations (concentrations up to 5  $\mu$ M were investigated); the observed CP binding was in some cases even higher than the binding to the corresponding MIP (Fig. S4 in Supplementary Material).

As discussed above, the optimal binding of

penicillin G to MIP1 was obtained in pure acetonitrile and the binding decreased with added water (Fig. 1). Comparison of a binding isotherm obtained in pure acetonitrile (Fig. 3a) with an isotherm obtained in acetonitrile containing 1% of water (Fig. 3b) confirmed that water had a detrimental effect on the specific binding to MIP1. The binding to the CPs was low in both cases. Binding isotherms in acetonitrile containing 1% of 10 mM aqueous salt solutions were determined by varying both the cation (Fig. 3c) and the anion (Fig. 3d). The corresponding isotherms obtained on CP1 showed lower binding than those obtained on MIP1 in all of the incubation mixtures (the CP isotherms are available in Fig. S6, Supplementary Material). The salts affected the binding in the order NaCl  $\approx$  NaBr  $\approx$  NaI > KCl > CsCl. Hence, the cation had a significant influence on the binding capacity and the effect followed the Hofmeister series whereas not much difference in binding was seen when the anion was varied. Average pKa values of polymers made from methacrylic acid have been reported to lie in the range 6.4–6.5 [39,40]. At neutral pH, the polymer has a net negative charge. Repulsion of anions from the polymer surface is a plausible explanation why no anion effect was observed.

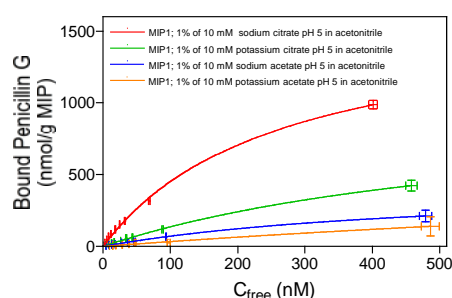
The binding of propranolol shows a different behavior than the binding of penicillin G in water-poor media as discussed above (Fig. 1). The explanation lies in the different binding mechanisms. The binding of propranolol at neutral pH has previously been attributed to an ion exchange mechanism between the negatively charged MIP and the protonated propranolol molecules [16]. Addition of competing salt



**Fig. 3** (a) and (b) Binding isotherms of penicillin G to MIP1 (red filled circles, red solid line) and CP1 (blue open circles, blue solid line) in (a) acetonitrile and (b) acetonitrile containing 1% of water. The green dotted lines show the specific binding obtained by subtracting the CP binding from the MIP binding; (c) Binding isotherms of penicillin G to MIP1 in acetonitrile containing 1% of 10 mM solutions of NaCl (red), KCl (green) or CsCl (blue); and (d) Binding isotherms of penicillin G to MIP1 in acetonitrile containing 1% of 10 mM solutions of NaCl (red), NaBr (blue), or NaI (green). MIP1 was imprinted with the sodium salt of penicillin G. Data points are the mean values of five replicates and standard errors are indicated with error bars.

ions caused a decrease in binding to MIP2 (Fig. S7 in Supplementary Material). The binding to CP2 decreased even more under these conditions, indicating that much of the MIP binding in water-poor media was due to non-specific ionic interactions. The net effect of salt addition was an increase in the specific binding to the MIP. However, the specific binding approached the same level in all of the mixtures tested and did not appear to follow the Hofmeister series of the ions present. Hence, the added salt ions played here another role than on MIP1. At the larger salt concentrations applied in pure aqueous media, however, the Hofmeister effect played a role on MIP2 and CP2 as discussed above (Fig. 2b).

The pH of the rebinding medium is known to affect the rebinding to MIPs as discussed in the introduction. In light of the large ion effects observed previously in this study, we suspected that not only the pH but also the choice of the buffer salt might affect the binding capacity. The influence of the pH on the binding of penicillin G was first screened in acetonitrile containing 1% of buffer of pH 4–7 (Fig. S8 in Supplementary Material). All buffers were prepared from the same buffer salt (i.e., sodium citrate). The rebinding to the MIP was higher in the buffered acetonitrile mixtures than in the non-buffered acetonitrile–water (99:1) mixture and approached the level observed in pure (non-aqueous) acetonitrile. Optimal binding was obtained at pH 5–6. The influence of different buffer salts at a fixed pH was then investigated. Binding isotherms in acetonitrile containing 1% of 10 mM buffers of pH 5 (either sodium citrate, potassium citrate, sodium acetate, or potassium acetate; Fig. 4) showed that (i) citrate buffers resulted in higher binding to the MIP than acetate buffers and (ii) sodium applied as the counterion gave higher binding than potassium.

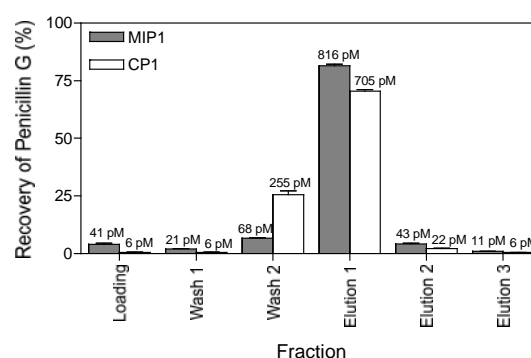


**Fig. 4** Binding isotherms of penicillin G to MIP1 in acetonitrile containing 1% of a 10 mM buffer pH 5 made from sodium citrate (red), potassium citrate (green), sodium acetate (blue), or potassium acetate (orange). MIP1 was imprinted with the sodium salt of penicillin G. Data points are the mean values of five replicates and standard errors are indicated with error bars.

In a Hofmeister series of anions, citrate is more kosmotropic than acetate and among the cations, sodium is more kosmotropic than potassium. Hence, the binding to the MIP followed the Hofmeister series with regard to both the cation and the anion at pH 5. At this pH, the carboxylic acid groups of the MIP are

mainly protonated, providing a neutral character to the polymer. The anions were therefore not repelled from the polymer surface, as was the case at neutral pH. The binding of the template to the MIP in the acetonitrile–acetate buffer mixtures were mainly of non-specific nature as evidenced by almost identical, or higher, binding to the CP (the CP isotherms are available in Fig. S9, Supplementary Material).

It is noteworthy that the presence of sodium citrate, NaCl, NaBr, or NaI in the aqueous portion of the rebinding mixture increased the binding to almost the same level as the one obtained in water-free acetonitrile. To demonstrate that insights into the Hofmeister effect can be used in a practical application to manipulate the binding to a MIP, a procedure for solid-phase extraction (SPE) of penicillin G from milk was developed (Fig. 5).



**Fig. 5** Solid-phase extraction of penicillin G from milk on MIP1 (filled bars) and CP1 (open bars). Bars show the recovery of penicillin G in eluted fractions and the estimated concentrations are shown above each bar. Loading and elution conditions are detailed in the experimental section.

Milk was spiked with radioactive penicillin G to a concentration of 10 nM (corresponding to 3.3 ppb; the MRL of penicillin G in milk is 4 ppb) [32]. The milk sample was diluted with a sodium citrate buffer of pH 5 containing 4 M NaCl (the final NaCl concentration in the diluted sample was 3.6 M) to promote binding to the MIP. Precipitated proteins and fat were removed by centrifugation. SPE columns packed with MIP1 and CP1 were equilibrated with the same buffer as the one used for the dilution of the sample (hereafter referred to as the dilution buffer). The soluble milk preparation was applied to the columns. Under these conditions, penicillin G was retained on both MIP1 and CP1. The columns were washed with the dilution buffer and then dried in vacuum. In a second wash with acetonitrile containing 1% of sodium citrate buffer of pH 5, conditions promoting specific binding were introduced. At this stage, penicillin G was partly eluted from CP1 whereas only a small fraction was eluted from MIP1. In the next steps, penicillin G was released from the columns by elution with methanol. The recoveries from the MIP and the CP were approximately 87 and 73%, respectively.

## CONCLUSIONS

This study has highlighted the influence of the Hofmeister effect on the binding to MIPs and CPs. Ionic species present in the aqueous rebinding media were demonstrated to affect the binding of both penicillin G and propranolol. In 100% aqueous media, the influence of salt ions followed the Hofmeister series with the more kosmotropic ions exerting the highest binding augmentation. The binding of penicillin G to MIP1 appeared to rely on hydrogen bonds since addition of small amounts of water had a negative effect on the binding capacity. MIP2 was much less sensitive to water and retained the template even at high amounts of water present in the rebinding solution. In water-poor media with salt ions added, the binding to MIP1 followed the Hofmeister series of the ions present while an ion exchange mechanism was seen on MIP2. The results support the theory that kosmotropic salts ions exert their effect through alteration of the hydrating environment [28]. When hydrogen bonding was crucial for recognition, as in MIP1, the kosmotropic ions appeared to promote the formation of stable MIP-template interactions. The findings of this study were used to develop a procedure for molecularly imprinted solid-phase extraction (MISPE) of penicillin G from milk.

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## SUPPLEMENTARY MATERIAL AVAILABLE

Experimental details and results on BET analysis including data on surface areas, pore sizes, and pore volumes (Table S1), adsorption and desorption isotherms (Fig. S1), and distributions of pore volumes (Fig. S2 and S3); binding isotherms on MIP1 imprinted with the potassium salt (Fig. S4) and sodium salt (Fig. S5) of penicillin G; binding isotherms of penicillin G to MIP1 and CP1 in acetonitrile containing 1% of 10 mM solutions of NaCl, KCl, CsCl, NaBr, or NaI (Fig. S6); binding of propranolol to MIP2 and CP2 in water-poor media (Fig. S7); pH screening plot (Fig. S8); and binding isotherms of penicillin G to MIP1 and CP1 in acetonitrile containing 1% of 10 mM buffer pH 5 made from sodium citrate, potassium citrate, sodium acetate, or potassium acetate (Fig. S9).

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## SUPPLEMENTARY MATERIAL

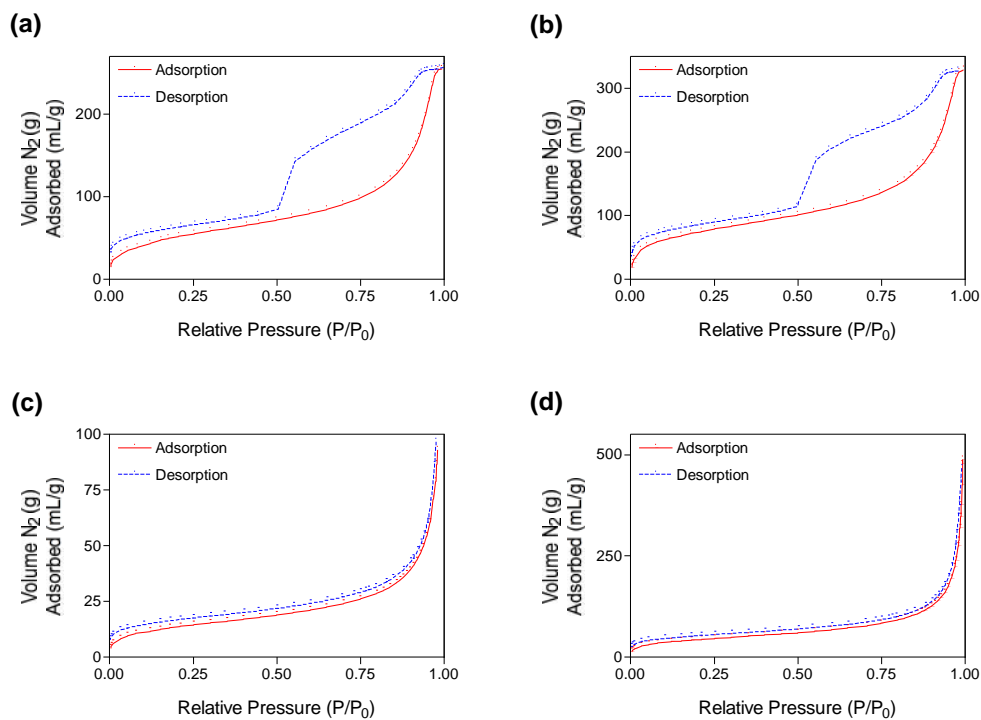
### BET analyses

Surface area and porosimetry were determined by nitrogen gas adsorption using an ASAP 2400 analyzer (Micromeritics Instrument Corp., Norcross, GA) after outgassing at 70 °C for 24 h. Calculations of the surface area were based on the BET (Brunauer, Emmett, and Teller) equation. The pore diameter and the pore volume were calculated by the method of Barrett, Joyner and Halenda (BJH). Similar BET isotherms were obtained for each MIP/CP pair (Fig. S1). All of the isotherms were of type IV with hysteresis, indicative of presence of mesopores [Sing, K.S.W. *Pure Appl. Chem.* **1982**, *54*, 2201–2218]. MIP1 and CP1 showed significantly higher hysteresis between the adsorption and the desorption branches at high pressures than at low pressures. The hysteresis of MIP2 and CP2 was of type H3. Surface areas, pore sizes, and pore volumes are summarized in Table S1 below. The pores sizes within each MIP/CP pair were similar while the surface areas and pore volumes were higher for the CPs than for the MIPs. The pore volume distributions are shown in Fig. S2 and S3.

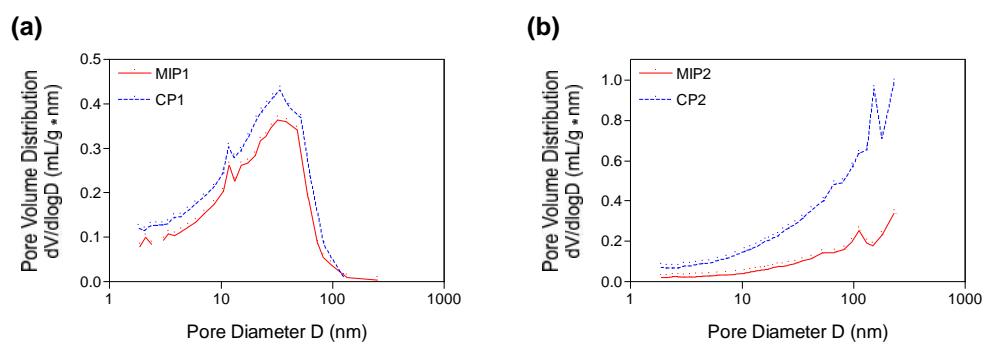
**Table S1.** Surface area, pore size, and pore volume of polymers.

Polymer	BET surface area (m <sup>2</sup> /g)	BJH adsorption average pore diameter (nm)	BJH desorption average pore diameter (nm)	BJH cumulative adsorption pore volume (mL/g)	BJH cumulative desorption pore volume (mL/g)
MIP1 <sup>a</sup>	195	11	6	0.350	0.390
CP1	272	10	6	0.433	0.469
MIP2	50	25	22	0.225	0.225
CP2	161	25	22	0.715	0.718

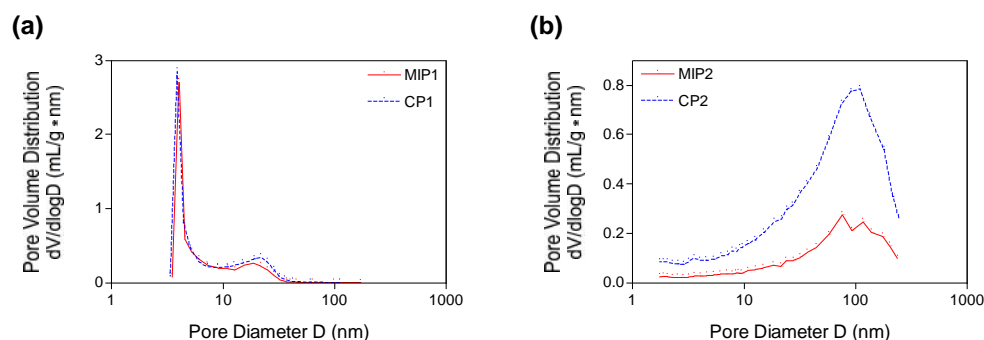
<sup>a</sup> Imprinted with the sodium salt of penicillin G.



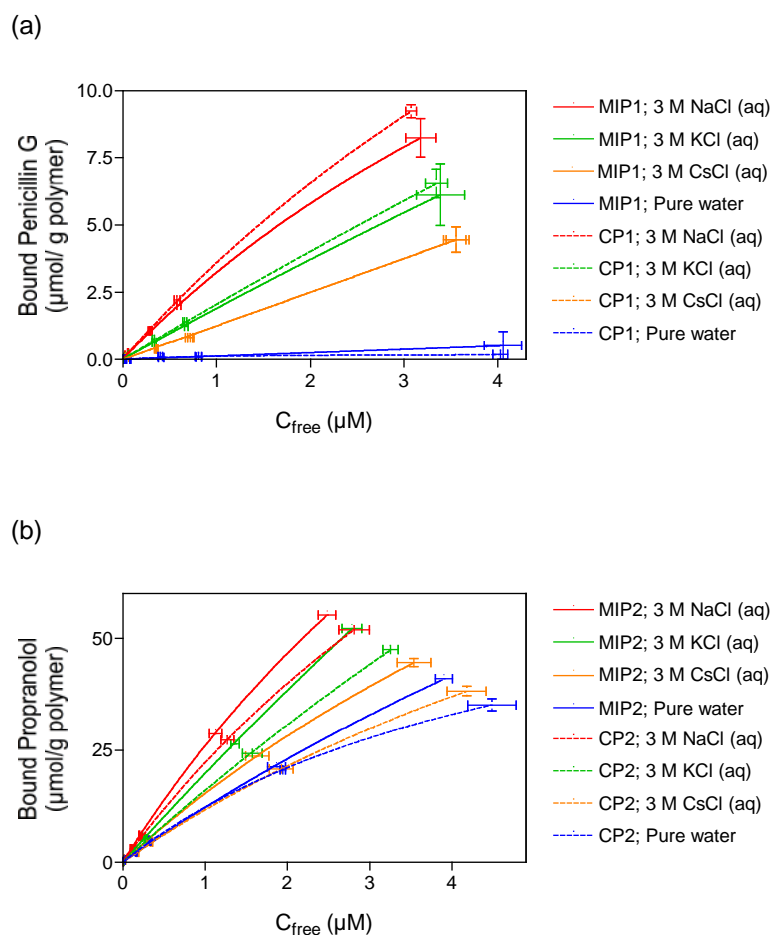
**Fig. S1** Adsorption- (filled circles, solid lines) and desorption isotherms (open circles, dotted lines) of  $N_2$  (g) on (a) MIP1; (b) CP1; (c) MIP2; and (d) CP2.



**Fig. S2** Distribution of the pore volume in MIP1 (filled circles, solid lines) and CP1 (open circles, dotted lines) calculated from (a) adsorption- and (b) desorption isotherms of  $N_2$  (g) by the BJH method.



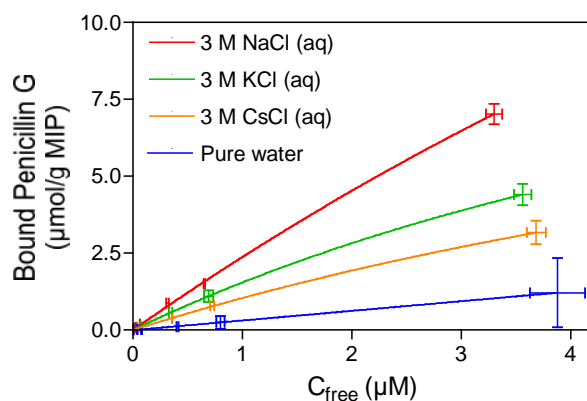
**Fig. S3** Distribution of the pore volume in MIP2 (filled circles, solid lines) and CP2 (open circles, dotted lines) calculated from (a) adsorption- and (b) desorption isotherms of  $N_2$  (g) by the BJH method.



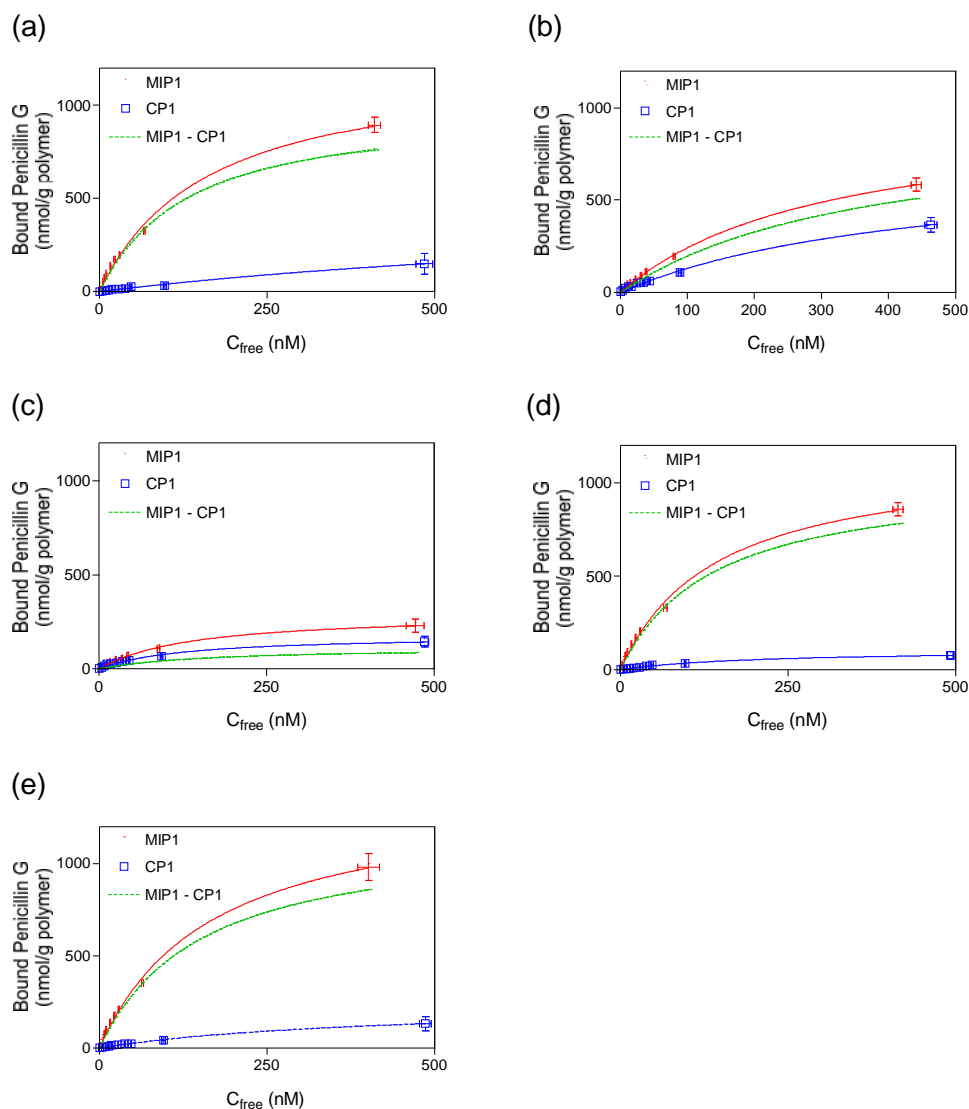
**Fig. S4** Binding isotherms of (a) penicillin G to MIP1 (imprinted with the potassium salt of penicillin G) and CP1 and (b) propranolol to MIP2 and CP2. MIP binding is indicated with filled circles and solid lines; CP binding is indicated with open circles and dotted lines. Binding was carried out in 3 M solutions of NaCl (red), KCl (green), CsCl (orange), or pure water (blue). Data points are the mean values of five replicates and standard errors are indicated with error bars.

### Binding isotherms on MIP1 imprinted with the sodium salt of penicillin G

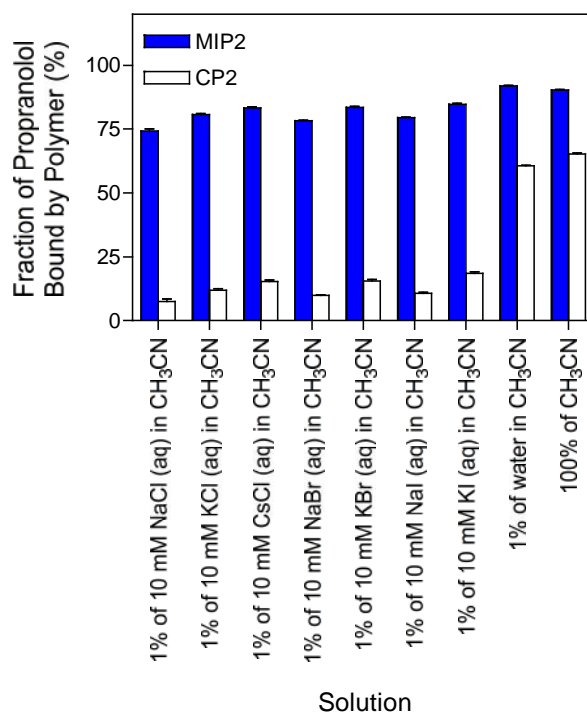
The isotherms shown in Fig. 2a and Fig. S4a were obtained on a MIP1 imprinted with the potassium salt of penicillin G. In order to investigate if the counter ion of the template affected the binding behavior of the resulting MIP, a MIP1 imprinted with the sodium salt of penicillin G was prepared. The binding isotherms to this MIP are shown in Fig. S5 below. The salts added to the incubation solution affected the binding to this MIP in the same order as on the MIP imprinted with the potassium salt of penicillin G (i.e., following the Hofmeister series).



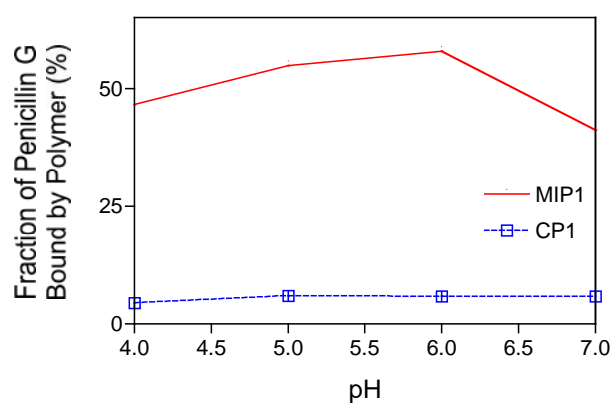
**Fig. S5** Binding isotherms of penicillin G to MIP1 imprinted with the sodium salt of penicillin G. Binding was carried out in either a 3 M solution of NaCl (red), KCl (green), CsCl (orange), or in pure water (blue). Data points are the mean values of five replicates and standard errors are indicated with error bars.



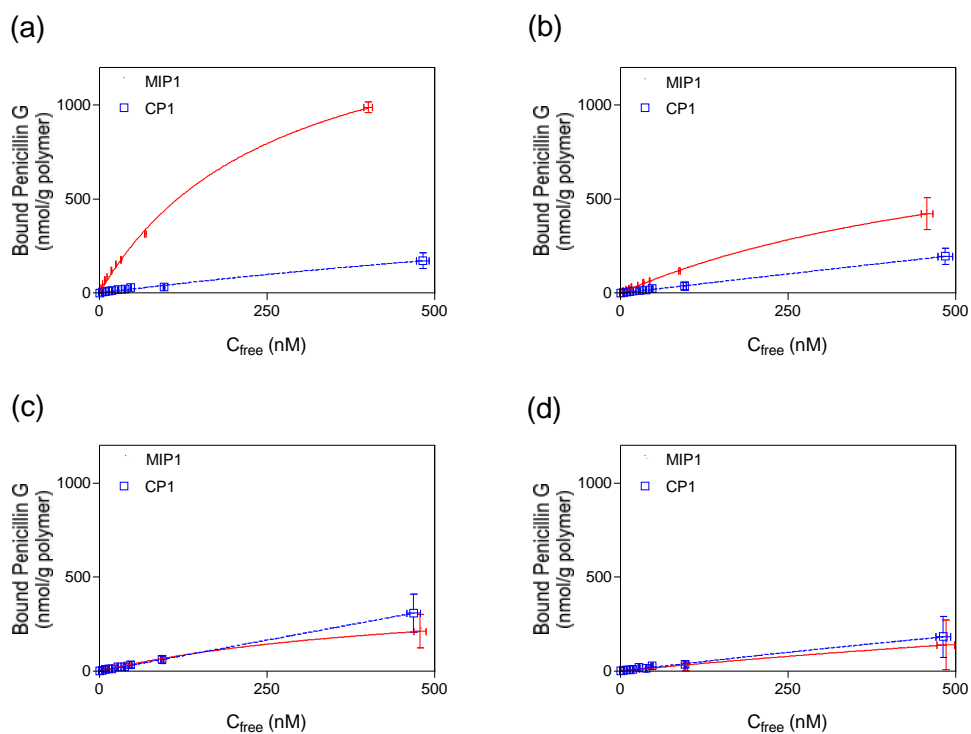
**Fig. S6** Binding isotherms of penicillin G to MIP1 (filled circles, solid lines) and CP1 (open squares, dotted lines) in acetonitrile containing 1% of 10 mM (a) NaCl; (b) KCl; (c) CsCl; and (d) NaBr; and (e) NaI. The green dotted lines show the specific binding obtained by subtracting the CP binding from the MIP binding. MIP1 was imprinted with the sodium salt of penicillin G. Data points are the mean values of five replicates and standard errors are indicated with error bars.



**Fig. S7** Binding of <sup>3</sup>H-labeled propranolol (1 nM) to MIP2 (filled bars) and CP2 (open bars) (0.1 mg/mL) in the indicated solutions. Data points are the mean values of five replicates and standard errors are indicated with error bars.



**Fig. S8** Binding of <sup>3</sup>H-labeled penicillin G (1 nM) to MIP1 (filled circles and solid line) and CP1 (open squares and dotted line) (1 mg/mL) in acetonitrile containing 1% of 10 mM sodium citrate buffer of the indicated pH. MIP1 was imprinted with the sodium salt of penicillin G. Data points are the mean values of five replicates and standard errors are indicated with error bars.



**Fig. S9** Binding isotherms of penicillin G to MIP1 (filled circles, solid lines) and CP1 (open squares, dotted lines) in acetonitrile containing 1% of 10 mM (a) sodium citrate pH 5; (b) potassium citrate pH 5; (c) sodium acetate pH 5; and (d) potassium acetate pH 5. MIP1 was imprinted with the sodium salt of penicillin G. Data points are the mean values of five replicates and standard errors are indicated with error bars.